

CJASN

CJASN's Renal Physiology for the Clinician

Providing an invaluable resource for practicing nephrologists and nephrology trainees

Leading investigators provide a focused update for practicing nephrologists and trainees on the latest developments in renal physiology within this comprehensive 18-part series available now in a user-friendly compiled pdf file.

Series Editors:

Mark L. Zeidel, MD, FASN

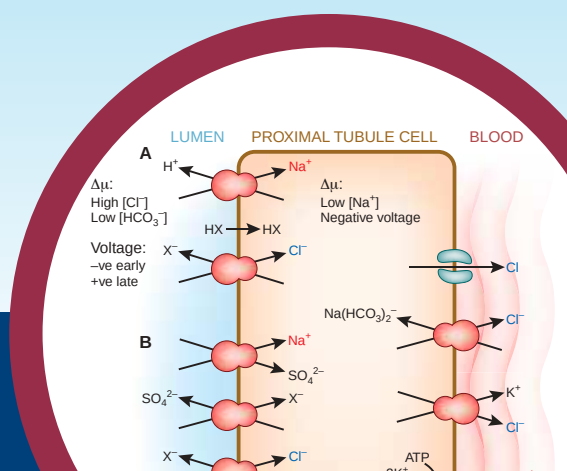
Melanie P. Hoenig, MD

Deputy Editor:

Paul M. Palevsky, MD, FASN

Editor-in-Chief:

Gary C. Curhan, MD, ScD, FASN



CJASN

Clinical Journal of the American Society of Nephrology

Renal Physiology for the Clinician

- Article 1** **A New CJASN Series: Renal Physiology for the Clinician**
Mark L. Zeidel, Melanie P. Hoenig, and Paul M. Palevsky
- Article 2** **Homeostasis, the Milieu Intérieur, and the Wisdom of the Nephron**
Melanie P. Hoenig and Mark L. Zeidel
- Article 3** **The Glomerulus: The Sphere of Influence**
Martin R. Pollak, Susan E. Quaggin, Melanie P. Hoenig, and Lance D. Dworkin
- Article 4** **Proximal Tubule Function and Response to Acidosis**
Norman P. Curthoys and Orson W. Moe
- Article 5** **Urine-Concentrating Mechanism in the Inner Medulla: Function of the Thin Limbs of the Loops of Henle**
William H. Dantzler, Anita T. Layton, Harold E. Layton, and Thomas L. Pannabecker
- Article 6** **Thick Ascending Limb of the Loop of Henle**
David B. Mount
- Article 7** **Distal Convoluted Tubule**
Arohan R. Subramanya and David H. Ellison
- Article 8** **Collecting Duct Principal Cell Transport Processes and Their Regulation**
David Pearce, Rama Soundararajan, Christiane Trimper, Ossama B. Kashlan, Peter M.T. Deen, and Donald E. Kohan
- Article 9** **Collecting Duct Intercalated Cell Function and Regulation**
Ankita Roy, Mohammad M. Al-bataineh, and Núria M. Pastor-Soler
- Article 10** **Control of Urinary Drainage and Voiding**
Warren G. Hill
- Article 11** **Integrated Control of Na Transport along the Nephron**
Lawrence G. Palmer and Jürgen Schnemann
- Article 12** **Osmotic Homeostasis**
John Danziger and Mark L. Zeidel
- Article 13** **Regulation of Potassium Homeostasis**
Biff F. Palmer
- Article 14** **Renal Control of Calcium, Phosphate, and Magnesium Homeostasis**
Judith Blaine, Michel Chonchol, and Moshe Levi
- Article 15** **Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion**
I. David Weiner, William E. Mitch, and Jeff M. Sands
- Article 16** **Chemical and Physical Sensors in the Regulation of Renal Function**
Jennifer L. Pluznick and Michael J. Caplan
- Article 17** **Physiology of the Renal Interstitium**
Michael Zeisberg and Raghu Kalluri

Article 18 Handling of Drugs, Metabolites, and Uremic Toxins by Kidney Proximal Tubule Drug Transporters

Sanjay K. Nigam, Wei Wu, Kevin T. Bush, Melanie P. Hoenig, Roland C. Blantz, and Vibha Bhatnagar

Article 19 Acid-Base Homeostasis

L. Lee Hamm, Nazih Nakhoul, and Kathleen S. Hering-Smith

CJASN

Clinical Journal of the American Society of Nephrology

Editors

Editor-in-Chief

Gary C. Curhan, MD, ScD, FASN
Boston, MA

Deputy Editors

Kirsten L. Johansen, MD
San Francisco, CA

Paul M. Palevsky, MD, FASN
Pittsburgh, PA

Associate Editors

Michael Allon, MD
Birmingham, AL

Jeffrey C. Fink, MD, MS, FASN
Baltimore, MD

Linda F. Fried, MD, MPH, FASN
Pittsburgh, PA

David S. Goldfarb, MD, FASN
New York, NY

Donald E. Hricik, MD
Cleveland, OH

Mark M. Mitsnefes, MD
Cincinnati, OH

Ann M. O'Hare, MD
Seattle, WA

Mark A. Perazella, MD, FASN
New Haven, CT

Vlado Perkovic, MBBS, PhD, FASN, FRACP
Sydney, Australia

Katherine R. Tuttle, MD, FACP, FASN
Spokane, WA

Sushrut S. Waikar, MD
Boston, MA

Section Editors

Attending Rounds Series Editor

Mitchell H. Rosner, MD, FASN
Charlottesville, VA

Education Series Editor

Suzanne Watnick, MD
Portland, OR

Ethics Series Editor

Alvin H. Moss, MD, FACP
Morgantown, WV

Public Policy Series Editor

Alan S. Kliger, MD
New Haven, CT

Renal Physiology Series Co-Editors

Mark L. Zeidel, MD, FASN
Boston, MA

Melanie P. Hoenig, MD
Boston, MA

Statistical Editors

Ronit Katz, DPhil
Seattle, WA

Robert A. Short, PhD
Spokane, WA

Editor-in-Chief, Emeritus

William M. Bennett, MD, FASN
Portland, OR

Managing Editor

Shari Leventhal
Washington, DC

CJASN

Clinical Journal of the American Society of Nephrology

Editorial Board

Rajiv Agarwal
Indianapolis, Indiana

Ziyad Al-Aly
Saint Louis, Missouri

Charles Alpers
Seattle, Washington

Sandra Amaral
Philadelphia, Pennsylvania

Jerry Appel
New York, New York

Arif Asif
Miami, Florida

Mohamed Atta
Baltimore, Maryland

Joanne Bargman
Toronto, ON, Canada

Brendan Barrett
St. John's, NL, Canada

Srinivasan Beddhu
Salt Lake City, Utah

Jeffrey Berns
Philadelphia, PA

Geoffrey Block
Denver, Colorado

W. Kline Bolton
Charlottesville, Virginia

Andrew Bomback
New York, New York

Ursula Brewster
New Haven, Connecticut

Patrick Brophy
Iowa City, Iowa

Emmanuel Burdmann
São Paulo, Brazil

Kerri Cavanaugh
Nashville, Tennessee

Micah Chan
Madison, Wisconsin

Anil Chandraker
Boston, Massachusetts

David Charytan
Boston, Massachusetts

Michael Choi
Baltimore, Maryland

Michel Chonchol
Denver, Colorado

Steven Coca
New Haven, Connecticut

Andrew Davenport
London, United Kingdom

Ian de Boer
Seattle, Washington

Bradley Dixon
Iowa City, Iowa

Lance Dworkin
Providence, Rhode Island

Jeffrey Fadrowski
Baltimore, Maryland

Derek Fine
Baltimore, Maryland

Kevin Finkel
Houston, Texas

Steven Fishbane
Mineola, New York

John Forman
Boston, Massachusetts

Lui Forni
Worthing, United Kingdom

Barry Freedman
Winston Salem, North Carolina

Masafumi Fukagawa
Kanagawa, Japan

Susan Furth
Philadelphia, PA

Martin Gallagher
Sydney, Australia

Maurizio Gallieni
Milan, Italy

Ronald Gansevoort
Groningen, Netherlands

Amit Garg
London, ON, Canada

Michael Germain
Springfield, Massachusetts

Eric Gibney
Atlanta, Georgia

John Gill
Vancouver, BC, Canada

David Goldsmith
London, United Kingdom

Stuart Goldstein
Cincinnati, Ohio

Barbara Greco
Springfield, Massachusetts

Orlando Gutierrez
Birmingham, Alabama

Yoshio Hall
Seattle, Washington

Lee Hamm
New Orleans, Louisiana

Ita Heilberg
São Paulo, Brazil

Brenda Hemmelgarn
Calgary, AB, Canada

Jonathan Himmelfarb
Seattle, Washington

Eric Hoste
Gent, Belgium

Chi-yuan Hsu
San Francisco, California

T. Alp Ikizler
Nashville, Tennessee

Tamara Isakova
Chicago, Illinois

Meg Jardine
Sydney, Australia

Michelle Josephson
Chicago, Illinois

Bryce Kiberd
Halifax, BC, Canada

Greg Knoll
Ottawa, ON, Canada

Jay Koyner
Chicago, Illinois

Holly Kramer
Maywood, Illinois

Manjula Kurella Tamura
Palo Alto, California

Hiddo Lambers Heerspink
Groningen, Netherlands

Craig Langman
Chicago, Illinois

James Lash
Chicago, Illinois

Eleanor Lederer
Louisville, Kentucky

Andrew Lewington
Leeds, United Kingdom

Orfeas Liangos
Coburg, Germany

Fernando Liaño
Madrid, Spain

John Lieske
Rochester, Minnesota

Kathleen Liu
San Francisco, California

Randy Luciano
New Haven, Connecticut

Jicheng Lv
Beijing, China

Mark Marshall
Auckland, New Zealand

William McClellan
Atlanta, Georgia

Anita Mehrotra
New York, New York

Rajnish Mehrotra
Seattle, Washington

Michal Melamed
Bronx, New York

Sharon Moe
Indianapolis, Indiana

Barbara Murphy
New York, New York

Nader Najafian
Boston, Massachusetts

Andrew Narva
Bethesda, Maryland

Sankar Navaneethan
Cleveland, Ohio

Alicia Neu
Baltimore, Maryland

Thomas Nickolas
New York, New York

Toshiharu Ninomiya
Fukuoka, Japan

Rainer Oberbauer
Vienna, Austria

Gregorio Obrador
Mexico

Runolfur Palsson
Reykjavik, Iceland

Mandip Panesar
Buffalo, New York

Neesh Pannu
Edmonton, Canada

Rulan Parekh
Baltimore, Maryland

Uptal Patel
Durham, North Carolina

Aldo Peixoto
West Haven, Connecticut

Anthony Portale
San Francisco, California

Jai Radhakrishnan
New York, New York

Mahboob Rahman
Cleveland, Ohio

Dominic Raj
Washington, District of Columbia

Peter Reese
Philadelphia, Pennsylvania

Giuseppe Remuzzi
Bergamo, Italy

Mark Rosenberg
Minneapolis, Minnesota

Andrew Rule
Rochester, Minnesota

Jeffrey Saland
New York, New York

Jane Schell
Pittsburgh, Pennsylvania

Bernd Schröppel
Ulm, Germany

Stephen Seliger
Baltimore, Maryland

Michael Shlipak
San Francisco, California

Edward Siew
Nashville, Tennessee

Theodore Steinman
Boston, Massachusetts

Peter Stenvinkel
Stockholm, Sweden

Harold Szerlip
Augusta, Georgia

Eric Taylor
Boston, Massachusetts

Ashita Tolwani
Birmingham, Alabama

James Tumlin
Chattanooga, Tennessee

Mark Unruh
Albuquerque, New Mexico

Raymond Vanholder
Gent, Belgium

Anitha Vijayan
St. Louis, Missouri

Ron Wald
Toronto, ON, Canada

Michael Walsh
Hamilton, ON, Canada

Matthew Weir
Baltimore, Maryland

Steven Weisbord
Pittsburgh, Pennsylvania

Jessica Weiss
Portland, Oregon

Adam Whaley-Connell
Columbia, Missouri

Colin White
Vancouver, BC, Canada

Mark Williams
Boston, Massachusetts

Alexander Wiseman
Aurora, Colorado

Jay Wish
Indianapolis, IN

Myles Wolf
Chicago, Illinois

Jerry Yee
Detroit, Michigan

Bessie Young
Seattle, Washington

Eric Young
Ann Arbor, Michigan

Carmine Zoccali
Reggio Calabria, Italy

Executive Director

Tod Ibrahim

Director of Communications

Robert Henkel

Managing Editor

Shari Leventhal

The American Society of Nephrology (ASN) marks 50 years of leading the fight against kidney diseases in 2016. Throughout the year, ASN will recognize kidney health advances from the past half century and look forward to new innovations in kidney care. Celebrations will culminate at ASN Kidney Week 2016, November 15–20, 2016, at McCormick Place in Chicago, IL.

www.cjasn.org

Submit your manuscript online through Manuscript Central at <http://mc.manuscriptcentral.com/cjasn>.

Contacting CJASN

Correspondence regarding editorial matters should be addressed to the Editorial Office.

Editorial Office

Clinical Journal of the American Society of Nephrology
1510 H Street, NW, Suite 800
Washington, DC 20005
Phone: 202-503-7804; Fax: 202-478-5078
E-mail: sleventhal@cjasn.org

Contacting ASN

Correspondence concerning business matters should be addressed to the Publishing Office.

Publishing Office

American Society of Nephrology
1510 H Street, NW, Suite 800
Washington, DC 20005
Phone: 202-640-4660; Fax: 202-637-9793
E-mail: email@asn-online.org

Membership Queries

For information on American Society of Nephrology membership, contact Pamela Gordon at 202-640-4668; E-mail: pgordon@asn-online.org

Subscription Services

ASN Journal Subscriptions
1510 H Street NW, Suite 800
Washington, DC 20005
Phone: 202-557-8360; Fax: 202-403-3615
E-mail: bhenkel@asn-online.org

Commercial Reprints/ePrints

Hope Robinson
Sheridan Content Services
The Sheridan Press
450 Fame Avenue
Hanover, Pennsylvania 17331
Phone: 800-635-7181, ext. 8065
Fax: 717-633-8929
E-mail: hrobinson@tsp.sheridan.com

Indexing Services

The Journal is indexed by NIH NLM's PubMed MEDLINE; Elsevier's Scopus; and Thomson Reuters' Science Citation Index Expanded (Web of Science), Journal Citation Reports - Science Edition, Research Alert, and Current Contents/Clinical Medicine.

Display Advertising

The Walchli Tauber Group
2225 Old Emmorton Road, Suite 201
Bel Air, MD 21015
Mobile: 443-252-0571
Phone: 443-512-8899 *104
E-mail: kim.boyd@wt-group.com

Classified Advertising

The Walchli Tauber Group
2225 Old Emmorton Road, Suite 201
Bel Air, MD 21015
Phone: 443-512-8899 *106
E-mail: rhonda.truitt@wt-group.com

Change of Address

The publisher must be notified 60 days in advance. Journals undeliverable because of incorrect address will be destroyed. Duplicate copies may be obtained, if available, from the Publisher at the regular price of a single issue.

Disclaimer

The statements and opinions contained in the articles of *The Clinical Journal of the American Society of Nephrology* are solely those of the authors and not of the American Society of Nephrology or the editorial policy of the editors. The appearance of advertisements in the Journal is not a warranty, endorsement, or approval of the products or services advertised or of their effectiveness, quality, or safety. The American Society of Nephrology disclaims responsibility for any injury to persons or property resulting from any ideas or products referred to in the articles or advertisements.

The Editor-in-Chief, Deputy, Associate, and Series Editors, as well as the Editorial Board disclose potential conflicts on an annual basis. This information is available on the CJASN website at www.cjasn.org.

POSTMASTER: Send changes of address to Customer Service, *CJASN Clinical Journal of the American Society of Nephrology*, 1510 H Street, NW, Suite 800, Washington, DC 20005. *CJASN Clinical Journal of the American Society of Nephrology*, ISSN 1555-9041 (Online: 1555-905X), is an official journal of the American Society of Nephrology and is published monthly by the American Society of Nephrology. Periodicals postage at Washington, DC, and at additional mailing offices. Subscription rates: domestic individual \$438; international individual, \$588; domestic institutional, \$970; international institutional, \$1120; single copy, \$75. To order, call 504-942-0902. Subscription prices subject to change. Annual dues include \$33 for journal subscription. Publications mail agreement No. 40624074. Return undeliverable Canadian addresses to PO Box 503 RPO West Beaver Creek Richmond Hill ON L4B 4R6. Copyright © 2016 by the American Society of Nephrology.

∞ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper), effective with January 2006, Vol. 1, No. 1.



A New *CJASN* Series: Renal Physiology for the Clinician

Mark L. Zeidel,* Melanie P. Hoenig,* and Paul M. Palevsky†

Clin J Am Soc Nephrol 9: 1271, 2014. doi: 10.2215/CJN.10191012

Introduction

With this issue, *CJASN* begins a new series of review articles designed to reconnect clinical nephrologists and trainees with the fundamentals of renal physiology and pathophysiology. For many of us, our initial interest in nephrology was the result of fascination with clinical fluid and electrolyte disturbances and fascination with the intricate underlying pathophysiologic mechanisms. However, in modern nephrology practice and training, several factors reduce the familiarity of practitioners and fellows with the fundamentals of renal physiology that initially piqued their interest. Increasingly, nephrology consultation in hospitals focuses on the management of AKI or the inpatient management of patients with CKD and ESRD, with fewer consults for electrolyte and acid-base disturbances. Moreover, the intensity of inpatient practice and outpatient care of CKD and ESRD patients often makes it difficult to focus on physiologic mechanisms, even when nephrologists are called on to help manage electrolyte and acid-base disorders. Finally, modern research training of renal fellows does not lend itself to the development of an in-depth understanding of renal physiology. In earlier eras, renal fellows were likely to perform research involving isolated perfused tubules, micropuncture, or other model systems, which emphasized renal physiology. In the current era, there is a greater emphasis on clinically oriented research and a decreased emphasis on basic physiology. Those nephrologists who embark on basic research often focus intently on detailed molecular pathways or genetic studies, which do not emphasize the systems physiology of renal homeostasis (1).

The renal community has made several efforts to reconnect clinicians and trainees with physiology. These efforts included the “Milestones in Nephrology” series, which ran from 1997 to 2001 in the *Journal of the American Society of Nephrology*, didactic and scientific sessions at every one of our national and international meetings, and for renal fellows, the National Course for Renal Fellows: The Origins of Renal

Physiology, which is held annually at the Mount Desert Island Biologic Laboratories, near Acadia National Park in Maine (1).

With this series, we seek to answer the question posed originally by Claude Bernard in the mid 1800s (2): “How does the kidney maintain the constancy of the internal milieu?” How does the kidney maintain constant serum osmolality, potassium, pH, calcium, and overall volume in the face of constant environmental challenges? We have invited a truly distinguished group of renal physiologists to address this overall question, starting with review articles on the control of glomerular filtration and segment by segment tubular function, and ending with articles describing the integrative function of the kidney in achieving homeostasis. The reviews will be brief but comprehensive, and, therefore, they will be accessible to practicing nephrologists, clinician educators, and trainees, but of sufficient heft to provide a focused review for renal physiologists. To enhance clarity, we will try to use a single visual vocabulary for diagrams of tubules and glomerular cells to make sure that the illustrations are consistent across the different review articles in the series. We hope that these reviews will be helpful to practitioners and trainees and useful as they teach physiology to the next generation of residents and medical students.

Disclosures

None.

References

1. Zeidel M, Bonventre J, Forrest J, Sukhatme V: A national course for renal fellows: The origins of renal physiology. *J Am Soc Nephrol* 19: 649–650, 2008
2. Bernard C: *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux*, Paris, J-B Baillière, 1878

Published online ahead of print. Publication date available at www.cjasn.org.

*Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts; and
†Renal Section, VA Pittsburgh Healthcare System, and Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Correspondence:

Dr. Mark L. Zeidel, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215. Email: mzeidel@bidmc.harvard.edu



Homeostasis, the Milieu Intérieur, and the Wisdom of the Nephron

Melanie P. Hoenig and Mark L. Zeidel

Abstract

The concept of homeostasis has been inextricably linked to the function of the kidneys for more than a century when it was recognized that the kidneys had the ability to maintain the “internal milieu” and allow organisms the “physiologic freedom” to move into varying environments and take in varying diets and fluids. Early ingenious, albeit rudimentary, experiments unlocked a wealth of secrets on the mechanisms involved in the formation of urine and renal handling of the gamut of electrolytes, as well as that of water, acid, and protein. Recent scientific advances have confirmed these prescient postulates such that the modern clinician is the beneficiary of a rich understanding of the nephron and the kidney’s critical role in homeostasis down to the molecular level. This review summarizes those early achievements and provides a framework and introduction for the new *CJASN* series on renal physiology.

Clin J Am Soc Nephrol 9: 1272–1281, 2014. doi: 10.2215/CJN.08860813

Division of
Nephrology, Beth
Israel Deaconess
Medical Center,
Harvard Medical
School, Boston,
Massachusetts

Correspondence:
Dr. Melanie P. Hoenig,
Division of
Nephrology,
Department of
Medicine, Beth Israel
Deaconess Medical
Center Clinic, Fa 8/
185 Pilgrim Road,
Boston, MA 02215.
Email: MHOenig@
bidmc.harvard.edu

Introduction

Critical advances in our understanding of renal physiology are unfolding at a rapid pace. Yet, remarkably, the lessons learned from early crude measurements and careful study still hold true; indeed, classic articles still serve as the basis for introductory textbooks on renal physiology and provide a solid working knowledge to clinicians. Drawings with just a handful of transporters at each nephron segment, known for more than half a century, are sufficient to understand basic mechanisms of autoregulation, clearance, and the effects of diuretics—the tools needed to care for patients. Yet we clinicians also benefit from a treasure trove of subsequent scientific advances, which have given us a detailed and comprehensive understanding of how the kidney maintains stable body chemistries and volume balance.

The layers of complexity and the mysteries that continue to unravel make it difficult to stay abreast of current research. Still, the modern nephrologist is in good company. In 1959, a medical student wrote to Homer Smith, the uncontested patriarch of modern nephrology at the time, to inquire about his rectilinear depiction of the nephron (Figure 1) and why he failed to mention the counter current theory in his famous 1956 textbook, the *Principles of Renal Physiology* (1,2). Indeed, the structure of the loop of Henle had been well known since the mid-1800s, but the importance of that eponymous structure, the gradient that it generated, and its role in the final product urine was only just elucidated at the time of the student’s correspondence. Before this, Homer Smith felt that the hairpin turn was just a vestige of embryology. This student’s misjustice was a curiosity, rather than a criticism. Carefully framed questions have always served to

advance our understanding. In this overview, we will describe, all too briefly, the ingenious methods used by early investigators and the secrets they unlocked to help create the in-depth understanding of renal physiology and pathophysiology that we enjoy today. Additional details will follow in the new *CJASN* series of review articles on the physiology of the kidney.

The Milieu Intérieur and the Kidney’s Essential Role

In the early 1800s, Darwin’s Theory of Evolution combined with the recognition that the body chemistries of many disparate species were remarkably similar led Claude Bernard to develop his theory of the Milieu Intérieur: “The constancy of the internal environment is the condition of a free and independent existence” (3). By this, he meant that the ability of our ancestor organisms to leave the oceans required that they develop the ability to “carry the ocean with them” in the form of an internal ocean, bathing their cells constantly in fluids that resemble the very seas from which they evolved. This concept, although reminiscent of the notion of bodily humors (4,5), marked an enormous advance because Bernard described both the features of bodily fluids and the need to maintain that internal milieu. Maintenance of the internal milieu was first called the “wisdom of the body” by Starling (6), who recognized that organisms must maintain the constancy of this internal ocean despite great fluctuations in diet, fluid intake, and other environmental conditions. The term *homeostasis* was later coined by behaviorist Walter Cannon to describe the physiologic processes that, in aggregate, maintain the constancy of the internal

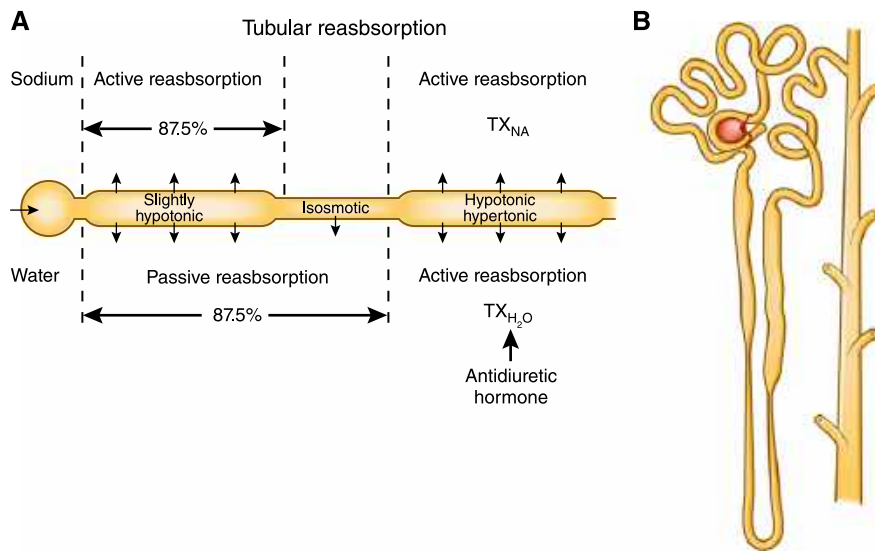


Figure 1. | Study of the kidney requires consideration of both the role of each segment and the three-dimensional architecture. (A) Homer Smith's rectilinear nephron mimics the straight trajectory of the fish nephron. (B) The human nephron is more intricate; the distal nephron greets its own glomerulus before it returns to the environs established by the loop of Henle. TX, treatment. A is modified from reference 1, with permission.

chemistries, as well as BP, body temperature, and energy balance (7).

The earliest insights into renal physiology came from the assiduous study of anatomy because, to a large degree, renal function follows structure. Meticulous drawings and histologic study of the animal and human kidney from William Bowman (8,9), Jacob Henle (10), and others, complete with capsule, capillaries, and convoluted tubules were available in Bernard's time, yet the mechanisms for the formation of urine and the kidney's role in homeostasis were not embraced until the next century. Until the 1920s, a debate raged on the mechanism of urine formation. Some researchers championed a filtration doctrine and others ascribed secretory power to both the glomerulus and tubules (11,12). The secretory theory was more popular, however, because the sheer volume of blood that would first need to be filtered and then reabsorbed by the kidney was enormous. Homer Smith noted that the filter-reabsorption strategy "seemed extravagant and physiologically complicated" (2).

Early Investigations Form the Framework

An interest in comparative physiology and the advent of the marine biologic laboratories that studded the Atlantic coast at the turn of the century helped frame an early understanding of the kidney and its role in evolution. Remarkably, increasingly complex fish with salty interiors adapted to fresh water, whereas amphibians rose from the sea to face the challenges mandated by scarce water. In his famed opus, Smith summarized the observations to date and waxed poetic (and philosophic), declaring, "Superficially, it might be said that the function of the kidneys is to make urine; but in a more considered view, one can say that the kidneys make the stuff of philosophy itself" (13). This impression, the sense of wonder at the intricate dealings of the kidney, permeates the scientific writings from that time to this day.

Studies performed in frogs, rabbits, and dogs in the early 1900s showed that the constituents of blood and urine differed because urine contained urea, potassium, and sodium salts, whereas blood contains protein, glucose, and very little urea. Furthermore, balance studies suggested that the volume and constituents of urine changed depending on changing intake or experimental infusions. In his "The Secretion of Urine" monograph published in 1917, Cushny summarized the available literature to date and described the brisk diuresis that followed sodium chloride infusions. He also reported findings that showed that the kidney produced acid urine in humans and the carnivora, whereas the herbivora had alkaline urine unless fed a protein diet (14). Despite this careful review and his presentation of the "modern view" that acknowledged that both filtration and secretion could exist, Cushny struggled with the data and felt that the theories were "diametrically opposed" because secretion and reabsorption would result in opposing currents along the renal epithelium. Clearly, methods were needed that could allow direct measurement of the filtrate and its modification along the nephron.

When Wearn and Richards introduced the micropuncture technique to the study of the kidney (first in amphibia, which had large renal structures amenable to manipulation), the debate on the formation of urine was resolved (11,15). By sampling the fluid elaborated from the glomerular capsule of a frog, the team demonstrated a protein-free filtrate that was otherwise similar to blood. By contrast, the frog bladder urine had a different composition from the blood and was free of glucose. These findings, in light of earlier data, supported the notion that urine is formed by glomerular filtration, and the urine is then modified in the tubules, by a combination of reabsorption and secretion. Subsequent studies by Walker and others inserted oil "plugs" or "blocks" in various segments of the nephron and distal to the sampling pipette so that the investigators could avoid contamination but still study urine from

different segments of the nephron and, therefore, characterize each segment's function, the ions absorbed, and the osmolarity of the fluid (16). These investigators then developed the "stop flow" technique in which they placed a pipette distal to the oil droplet, infused fluid into that segment, and then sampled the fluid at the end of that segment to determine how the fluid had been altered (Figure 2A) (17).

This meticulous work was confirmed in mammals by extension of the micropuncture technique to rat and guinea pig kidneys (Figure 2B). However, despite the ingenious use of oil blocks to prevent upstream tubular fluid from reaching downstream segments and then substituting artificial perfusates, micropuncture studies did not permit control of the composition of fluids on both sides of the tubular epithelium. This limitation was remedied after World War II, when Hans Ussing developed his famed chamber methods (Figure 3). With this strategy, transport across isolated epithelia could be studied quantitatively by systematically altering the ionic composition and voltages

of the solutions on either side of the epithelium (18). Careful transport studies using model epithelia from nonmammals, including the toad bladder, the turtle bladder, and the flounder bladder, which anatomically and functionally model collecting duct principal cells, collecting duct intercalated cells, and distal tubule cells, respectively, gave important insights into transport mechanisms in these segments. In the late 1960s, Burg and colleagues developed methods for isolating and perfusing individual mammalian nephron segments, first from rabbits and then from mice. These preparations, along with the ability to measure minute quantities of ions and volumes from these tubules with ion-specific electrodes, including the picapnotherm (which measures minute quantities of carbon dioxide), permitted investigators to examine in detail the mechanisms, driving forces, and regulation of transport across individual nephron segments (19). With painstaking effort, investigators dissected tubules, perfused the segment with fluid of specific ion concentrations, and collected the "waste" fluid from the other end of the segment (Figure 4).

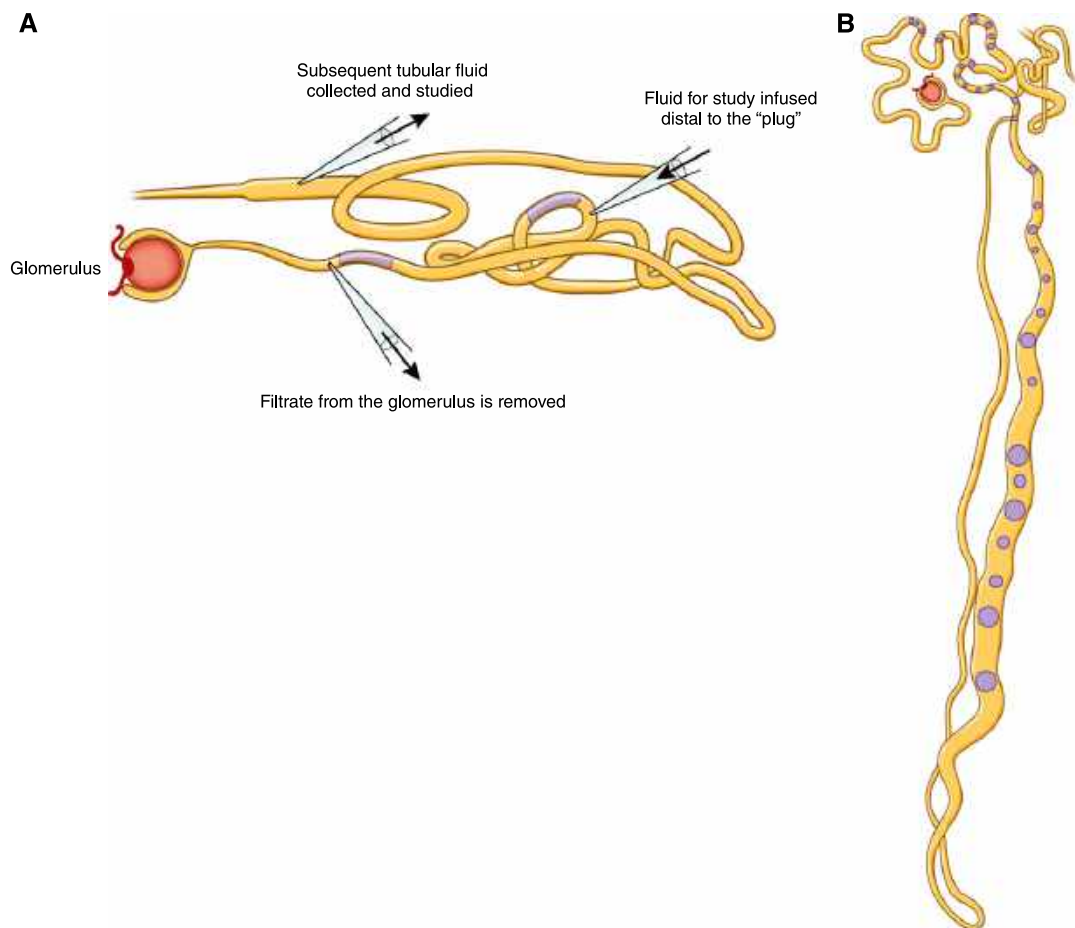


Figure 2. | Micropuncture and "stop flow" techniques were used to help define the role of each segment of the nephron. (A) The proximal tubule from the kidney of the aquatic salamander is illustrated here. A micropipette removes the filtrate at a point just proximal to a "plug" of mineral oil. To determine the role of the tubule in handling of individual constituents (reabsorption, secretion, or diffusion), fluid was injected into the tubule at different locations and then collected distally. This "artificial" fluid could be altered to differ from the normal filtrate by one or more constituents. (B) A sketch of a *camara lucida* drawing of a guinea pig nephron after microdissection (these drawings were created with the aid of a light projector because photomicrographs were not readily available at the time). Oil or mercury blocks could be inserted at various points along the nephron and fluid from the lumen could be collected and studied. A is modified from reference 17, with permission; B is modified from reference 16, with permission.

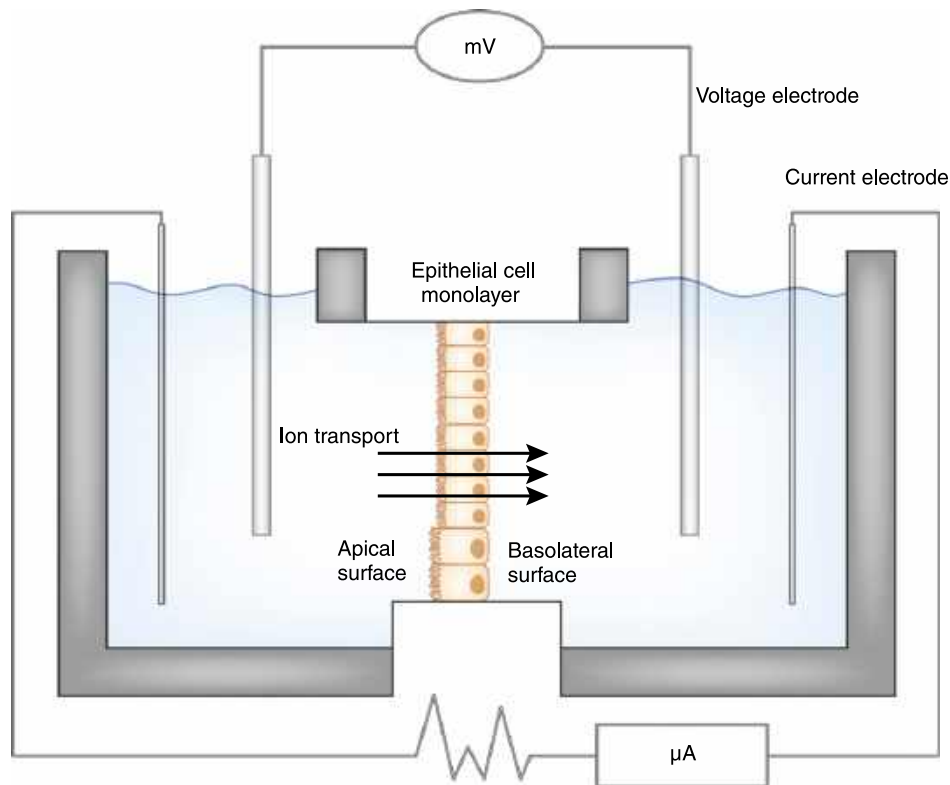


Figure 3. | The Ussing Chamber can be used to measure ion transport between the two sides of an epithelial cell membrane by polarized cells. Here, a monolayer of epithelial cells separates two compartments. Fluid in the two compartments is identical to eliminate the contribution of passive paracellular diffusion driven by differences in concentration, osmotic pressure, or hydrostatic pressure. Voltage electrodes placed near the epithelial membrane maintain the potential difference at zero so that the current measured by the current electrodes reflects the movement of ions by active transport through the epithelial cells.

This arrangement allowed investigation of individual segments of the nephron to better characterize the features of transport, electrochemical gradients, coupling with other ions, active versus passive transport, the threshold for reabsorption, and the permeability to water (1). The resulting flurry of studies, spanning nearly 2 decades, defined the phenomenology and regulation of transport, and identified, at least functionally, the transporter proteins responsible for homeostasis (20).

Meanwhile, in the clinical realm, the flame photometer became available in the late 1940s and this innovation made it possible to measure more than a dozen samples of blood for both sodium and potassium in under an hour. Before this time, electrolyte measurements were onerous and involved both chemical extractions and precipitations (21). Studies of electrolytes and the metabolic derangements were now possible; when this process was linked to an autoanalyzer that also provided chloride and total CO_2 , interest in acid-base disorders soared and the concept of “Gamblegrams” flourished (22). Dr. Gamble, a disciple of Henderson, studied a range of different insults from gastrointestinal losses to advanced CKD and their effect on electrolytes, and described the kidney as the “remarkable organ of regulation, the kidney sustains the chemical structure of extracellular fluid” (23).

Later, availability of the automatic analyzers also spurred a large literature using metabolic balance studies to characterize everything from bed rest or water immersion to the effects

of pharmacologic agents like chlorothiazide (24,25). In these detailed studies, investigators characterized vital signs, weight, intake, excretion, electrolytes, clearance, plasma volume, and hormonal levels. These data helped solidify the concepts of the steady state in homeostasis (26).

The stage was now set to identify specific renal transporters, describe how they function, and characterize how they are regulated. The remarkable reabsorptive task of the nephron tubules requires energy and active transport. It was not long after Nobel laureate Jens Skou’s 1957 discovery of the Na-K-ATPase in crab nerve microsomes (27,28) that this critical transporter was identified in the kidney. Because of its abundance, the enzyme was identified in crude homogenates of the renal cortex and medulla and Na-K-ATPase activity was later measured in individual segments of the nephron. The highest activity was in the thick ascending limb and distal convoluted tubule (DCT) and considerable activity was also observed in the proximal tubule. Further study helped identify the polarity of the renal epithelial cells with the Na-K-ATPase at the basolateral membrane. This finding helped solidify the concept that energy generated from this housekeeping enzyme, which maintains the normal cellular ion concentration, is harnessed by the kidney to reabsorb the bulk of the filtered sodium along with a host of other substances (29).

With a map in place for the role of each segment of the nephron and a solid understanding of factors that influence

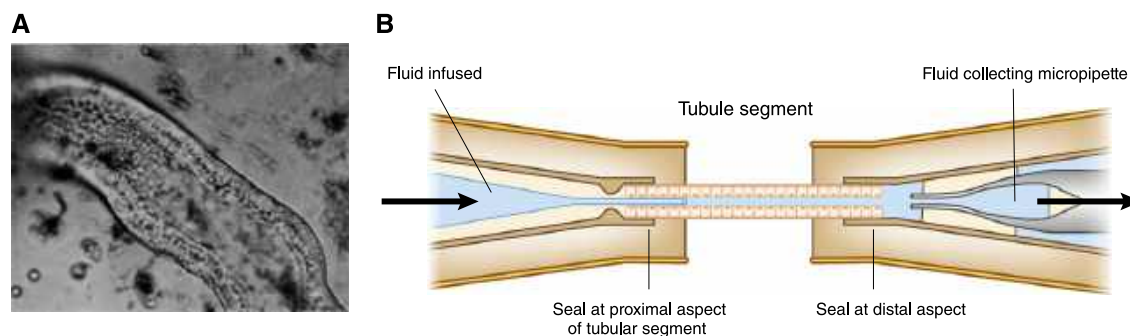


Figure 4. | Study of isolated perfused tubular segments allowed study of each of the different nephron segments independently. (A) A photomicrograph of a portion of a rabbit proximal convoluted tubule during perfusion. (B) A schematic diagram of the technique. One end of the dissected tubule was connected to a micropipette, which was used to perfuse the lumen, and the other end was connected to a collection micropipette. Both the luminal fluid and the peritubular fluid could be controlled to assess tubular transport characteristics. A is reprinted with permission from Burg MB: Perfusion of isolated renal tubules. *Yale J Biol Med* 45: 321–326, 1972.

the actions in those segments, research efforts by a multitude of investigators have set out to characterize the wide array of transporters in the nephron, their molecular structure, their distribution in the nephron, and their role in normal physiology and disease. This effort has been aided by sophisticated and rapidly evolving techniques with PCR, cloning and amplification of cDNAs, and expression in *Xenopus* oocytes, knockout mice, genome-wide association studies, and other models.

The Ultrastructure of the Glomerulus, the Concept of Clearance, and Autoregulation

Once the debate on the formation of urine was settled and it was clear that urine was formed by filtration, reabsorption, and secretion, mysteries of the elegant filtration design were explored and the concept of clearance was developed. Use of scanning electron microscopy allowed researchers to appreciate the three-dimensional structure of the cells and the ingenious design of the glomerulus. Podocytes were visualized extending their primary, secondary, and tertiary projections to interdigitate with neighboring “feet” and form the filtration slit diaphragm. Meanwhile, within the capillary bed, the delicate fenestrated endothelium drapes the basement membrane. A series of investigations into the dynamics of glomerular ultrafiltration, first in a unique strain of Wistar rats with surface glomeruli and later in primates, helped define factors that create the net driving force for filtration and provided the mathematical framework for our current understanding of these forces (30). In addition, studies on the permselectivity of the glomerular capillary wall using ferritin molecules of various sizes that were neutral, anionic, or cationic revealed that particles were restricted based on both charge and size; this explained the limited clearance of albumin at 39 Å, which is smaller than the 42 Å pores observed in the glomerular endothelial cell (31).

More recently, new techniques have helped further characterize these observations. For example, models of nephron development, such as the zebrafish, transparent and rapidly growing fish with a single pair of nephrons, have been indispensable to determining the effects of single defects on kidney function and development (32). Myriad

proteins that form the filtration slit diaphragm are characterized and defects in several of these proteins can predictably cause the nephrotic syndrome. Details of the complex meshwork of the basement membrane, a joint effort by the endothelial and epithelial cells, can result in thin basement membrane disease or hereditary syndromes, such as Alport syndrome. The elixir, vascular endothelial growth factor, appears to support the endothelium; when it is compromised, endothelial cells cannot sustain the regular challenges required to support normal structure and function of this unique capillary bed and, thus, thrombosis and endothelial injury can occur. In addition, direct micropuncture of glomeruli allowed a detailed description of normal glomerular hemodynamics and identified the derangements in glomerular function that occur with disease. Application of glomerular micropuncture in animals with glomerular damage caused by hypertension, diabetes, or loss of renal mass led directly to current therapies, including dietary protein restriction and use of angiotensin-converting enzyme inhibition.

The role of the glomerulus in “clearance” has provoked significant inquiry as well. Early clinical investigators explored the clearance of urea and creatinine after ingestion or determined the clearance with the infusion equilibrium methods, first with inulin and later with a host of radioactive markers, such as ^{125}I -iothalamate. When it became possible to measure the low concentrations of creatinine in serum, use of endogenous creatinine to calculate and later estimate clearance became possible. The pitfalls of this strategy (e.g., contribution from secretion, variability based on muscle mass, and the changes in serum levels related to diet and volume status, all of which make it less precise than the measured GFR) are more than balanced by the convenience (33).

It was soon evident that massive daily glomerular filtration could translate into life-threatening losses if there were no mechanisms in place to limit them in the event of low perfusion. Some researchers heralded these strategies as “acute renal success” (34); however, further study of the integrated response that maintains the GFR over a wide range of perfusion pressures provided an understanding of the roles of the juxtaglomerular apparatus in autoregulation and tubuloglomerular feedback (35).

Sodium and Water Homeostasis

Sodium, the major extracellular cation, plays a pivotal role in the maintenance of extracellular fluid volume and perfusion of vital organs and capillary beds. The kidney has an elaborate array of sodium transporters throughout the nephron (36). In the proximal tubule, it is linked to an elegant mechanism to reabsorb the filtered bicarbonate load by excreting H^+ ions with the electroneutral antiporters or Na^+/H^+ exchangers. Reabsorption of the ample filtered sodium also plays an important role in the reabsorption of glucose, sulfate, phosphate, and several amino acids. The remaining fraction of filtered sodium is reabsorbed with unique transporters in each of the subsequent nephron segments in which apical reabsorption of sodium is rate limiting. These transporters include the furosemide-sensitive channel in the loop of Henle, the thiazide-sensitive sodium chloride cotransporter that is primarily in the DCT, and the epithelial sodium channel transporter that is located primarily in the collecting tubules (Figure 5).

Our understanding of the mechanisms of sodium transport along the nephron comes from disparate sources. The advent of sulfonamides, investigated initially as much-needed antibiotics after World War II, were promptly recognized for their saluretic effects and soon revealed a wealth of secrets regarding the transport of sodium throughout the nephron (37). Genetic disorders also provided important clues. Endocrinologist Frederick Bartter and others described a set of youths afflicted with growth and mental retardation, muscle cramps, salt craving,

polyuria, and polydipsia. Bartter initially attributed his eponymous syndrome to a state of aldosterone excess with angiotensin resistance but when three-fourths adrenalectomy did not resolve the defect, he focused on the loop of Henle. Subsequent contributions from physiologists helped distinguish this disorder from Gitelman's syndrome and helped define the interplay of transporters in the loop of Henle and the DCT. However, it was the stunning characterizations by geneticists that helped identify mutations in several genes; these discoveries explained the subtle differences in the phenotype of these disorders and will be carefully considered within this series (38,39).

An endocrinopathy was also the initial theory that Dr. Liddle invoked to describe a family with early onset severe hypertension and hypokalemia that was notable for suppressed renin and aldosterone. As soon as the epithelial sodium channel was characterized, investigators demonstrated complete linkage in affected individuals with a defect in this transporter that resulted in a constitutively active sodium channel (40). Insights into the molecular biology, structure, function, and regulation of each of these sodium transporters has clearly enriched our grasp of renal physiology and complemented earlier predictions.

Hormonal and sympathetic nervous input can greatly augment sodium reabsorption, particularly by angiotensin II in the proximal tubule and aldosterone in the distal nephron, whereas the effect of atrial natriuretic peptide in the medullary collecting duct was found to be the opposite (41). Knowledge of these transporters is critical to the understanding of the

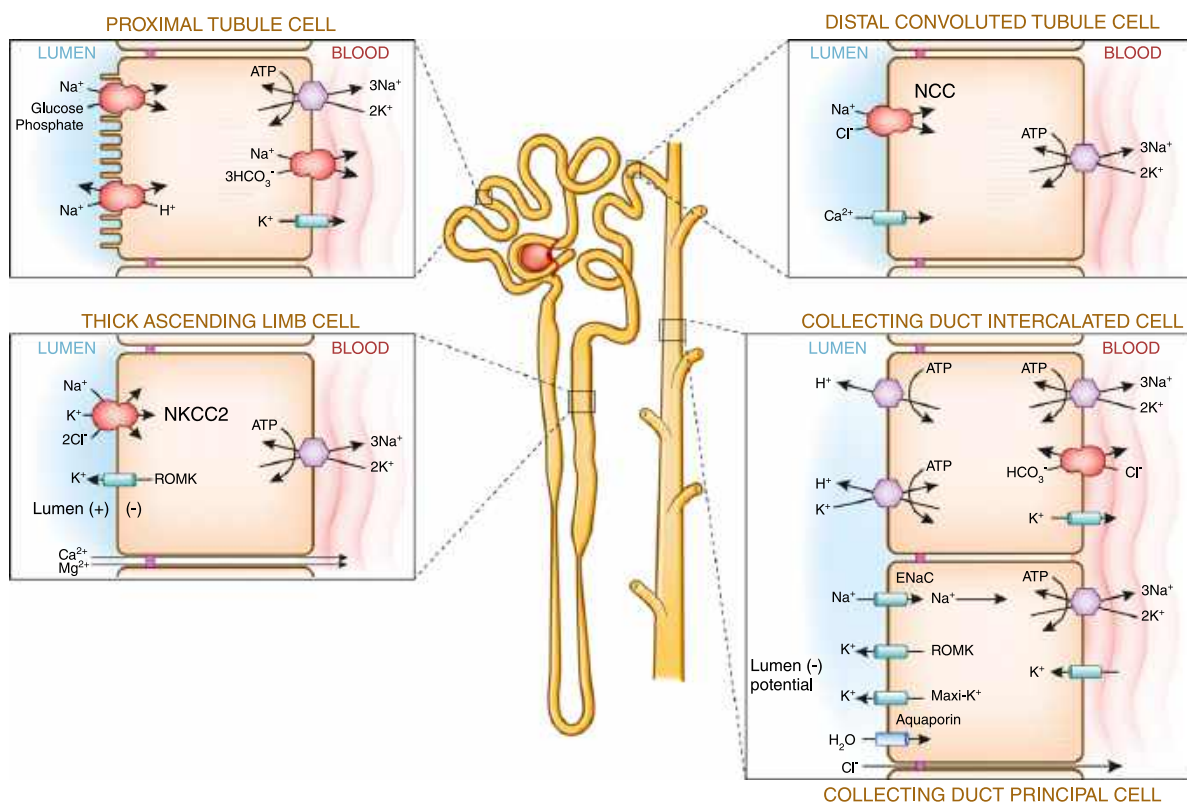


Figure 5. | The unique transporters and cell structure of each segment of the nephron work in concert to maintain homeostasis. ENaC, epithelial sodium channel; NKCC2, $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter; ROMK, renal outer medullary potassium.

clinical use of diuretics and the care of patients with a wide variety of issues, from the patient with essential hypertension to the complex patient with cirrhosis.

In the 1960s, Guyton proposed that all hypertension, ultimately, is a result of the failure of the kidney to excrete the excess of total body sodium with a normal pressure natriuresis (42). Although this theory has been disputed over the years, it is notable that monogenic defects that lead to hypertension or hypotension are found exclusively in genes that encode either renal transporter proteins or proteins that regulate the function of renal transporter proteins and ultimately renal sodium handling.

Despite mounting evidence on the importance of sodium in the kidney's role in homeostasis, investigators in the 1950s soon recognized that the measured serum sodium correlated poorly with the total body sodium by comparing these values in heterogeneous patients with a variety of chronic conditions. Instead, the serum sodium correlated well with the serum osmolality (particularly when corrections were made for the osmotic contributions of glucose and nonprotein nitrogen) (43). (Of note, the same investigators also recognized that the $\text{Na}^{2+}\text{-K}^{+}$ /total body water ratio correlated closely with "corrected" serum sodium and explained the importance for accounting of potassium repletion during the treatment of hyponatremia.) Maintenance of the plasma osmolality was noted to be tightly regulated by both the release of vasopressin and the kidneys' response (44). This interplay between the two is essential for water homeostasis, a critical factor in the maintenance of cell volume. Although cells have developed strategies to deal with excess or insufficient water, these volume regulatory changes require extrusion or inclusion of electrolytes, which alters the cellular interior milieu and wreaks havoc on normal cellular function. Later adaptations allow cells to return toward normalcy but only within a small range. Water reabsorption requires the ability to both establish an osmotic gradient in the kidney and to reabsorb water from the urinary filtrate. The kidney has an elegant strategy to concentrate or dilute the urine by its response to vasopressin and the ability to deploy aquaporins to the luminal membrane (45). At least seven aquaporin isoforms are expressed in the kidney and play important roles at different sites. In the proximal tubule and thin descending limb, aquaporin 1 appears to serve as the dominant gateway for water reabsorption, whereas trafficking of aquaporin 2 along cytoskeletal elements in the collecting duct cells allows reabsorption of water and urine concentration in the principal cells of the collecting ducts (46). Detailed study of the molecular structure and cell physiology of these transporters has allowed insight into the rare genetic diseases that affect aquaporins, such as congenital nephrogenic diabetes insipidus and the common acquired defects related to lithium, calcium, and even urinary obstruction. Similarly, study of the vasopressin receptor has resulted in new strategies and pharmacologic agents for the treatment of states of excess antidiuretic hormone and polycystic kidney disease.

Acid-Base Homeostasis

Maintenance of pH is a critical activity of the kidney and is essential for normal cellular function because the pH

dictates the charged state of proteins that affects conformational shape, enzymatic activity, binding, and cellular transport and, thus, allows proteins to perform essential metabolic functions. Although some acid-base enthusiasts enjoy consideration of the "strong ion difference" to reconcile data, the normal kidney's remarkable response to subtle differences in pH or, more likely, intracellular CO_2 does not take these differences into account. Although the exact mechanisms used to sense pH are still not yet understood, it is well known that the kidney plays a dominant role in the regulation of the acid-base balance (47). Indeed, with acidosis, a complex intracellular cascade ensues, including activation of the electroneutral sodium-coupled amino acid transporter for glutamine, an increase in glutamine metabolism and ammoniogenesis, as well as an increase in the expression of the sodium hydrogen antiporter (48,49). Additional orchestrated responses in the distal nephron with the help of the machinery in the intercalated cells and neighboring principal cells contribute to the kidneys' response to the challenges of acidemia (50).

By contrast, metabolic alkalosis can be easily and rapidly handled by the kidney by excretion of excess filtered bicarbonate or may be perpetuated by the kidney because of a response to volume depletion with secondary activation of the renin-angiotensin and aldosterone axis or from primary hyperaldosteronism. These processes are intimately linked to the regulation of potassium as well. Finally, the kidney responds to the challenges of primary respiratory disorders to offset the effects of these disturbances in a predictable fashion (51,52). In chronic respiratory alkalosis, the kidney can decrease acid excretion by decreasing ammonia production and bicarbonate retention; in respiratory acidosis, activity of the Na-H antiporter is augmented so that more bicarbonate is reabsorbed (53). Understanding of these complex processes helps in the care of actual patients who develop acid-base disorders from a wide variety of insults.

Potassium Homeostasis

Excretion of potassium in excess of the amount filtered is another triumph of the kidney in electrolyte homeostasis and its mechanism, another important milestone in the understanding of renal pathophysiology (54–56). Potassium, the major cation in the body, must be maintained in high concentrations in the intracellular space and a low concentration in the extracellular fluid to allow both normal cellular function and the considerable gradient required for excitation of nerves and contractions of muscles. The kidney plays its role by reabsorption of nearly all of the filtered load proximally and variable secretion in the distal nephron. Along the way, potassium is secreted into the lumen with the help of the renal outer medullary potassium transporter, which provides sufficient substrate to the NaKC2 transporter, and by the "big potassium," "maxi," or high conductance transporter (57). Both appear to play an important role in the secretion of potassium in the distal nephron dictated by the influence of aldosterone and magnitude of distal flow (58).

Divalent Cations and Phosphate Homeostasis

The kidney plays a critical role in maintaining both normal extracellular calcium ion levels and the vast repositories of

calcium needed for normal intracellular function and maintenance of the skeleton. Integrated control by parathyroid hormone and 1,25-dihydroxyvitamin D helps achieve this end (59). Calcium is reabsorbed through a paracellular route in the proximal tubule and the thick ascending limb, whereas there are unique, well characterized transient receptor potential ion channels in the DCT. Each of these regions is controlled by local effects prescribed by the calcium sensing receptor and modulated by the pH (60). There is also an interesting *pas de deux* between sodium and calcium handling. Volume depletion or decreased sodium delivery decreases urinary calcium either by increasing proximal reabsorption or by promoting reabsorption in the DCT with changes in the activity of the basolateral Na^+/Ca^+ exchanger or hyperpolarization of the luminal plasma membrane (61,62). The fate of magnesium homeostasis is intimately linked to that of other cations. In parallel to calcium, the majority of filtered magnesium is reabsorbed in the proximal tubule and thick ascending limb by paracellular movement mediated, in part, by the claudin proteins, which govern ion movement through the otherwise tight junctions in those regions (63). In addition, the action of the renal outer medullary potassium transporter to supply more potassium in the lumen for the NaKC2 transporter is thought to create a lumen-positive transepithelial potential difference that can favor cation reabsorption in this segment (64). By contrast, in the DCT, movement of Mg^{2+} is an active transcellular process. At this segment, cation channels from the melastin transient receptor potential subfamily play a major role in this endeavor, evidenced by Mg^{2+} wasting seen in the rare genetic disorders with defects in this channel (65).

Emerging details on phosphate metabolism have identified a family of sodium phosphate transporters that help reabsorb the bulk of the filtered phosphate in the proximal tubule (66). These transporters appear to be affected by a series of factors, including the recently characterized fibroblast growth factor 23 and its obligate coreceptor Klotho, which together, *via* the fibroblast growth factor receptor, inhibit the reabsorption of sodium-dependent phosphate reabsorption and lower vitamin D levels by downregulating the gene for 1α -hydroxylase (67). Phosphate that escapes proximal reabsorption and is delivered distally is available to bind H^+ as an important source of “titratable acid” (68).

Protein Metabolism

Even Richard Bright (69) in the 1820s recognized that in “dropsy” (or edema) of renal origin, urea was increased in the blood and decreased in the urine such that urea could serve as a marker of kidney failure. One hundred years later, Thomas Addis tried to assess renal function using urea clearance and “rest” the kidneys from the “work” of clearing proteins by prescribing a low-protein diet (70). Landmark studies by Brenner’s group suggested that this strategy was correct because high-protein diets fed to laboratory animals can be shown to increase renal blood flow and glomerular filtration and subsequently contribute to the progression of CKD (71). Nevertheless, the specific mediators that lead to hyperfiltration and contribute to the changes seen with a high-protein diet are yet to be determined.

Renal Physiology Has Significant Clinical Relevance

One of the considerable gifts to the field of nephrology is that there is a deep and growing understanding of the intricacies of kidney function and the ingenious methods that the kidney uses to govern homeostasis. These discoveries complement observations made with careful consideration and primitive measurements in the past. Predictions on the movement of ions have been translated by detailed characterization, on the molecular level, of ion transporters and provide insight into the integrated responses of the kidney to the maintenance of the internal milieu. Those of us who are privileged enough to care for patients with disorders of the kidney can utilize knowledge gleaned in the laboratory to understand real clinical concerns.

Because it is difficult for any practicing nephrologist to stay abreast of the rapidly unfolding revelations, this new renal physiology series will serve as an update to the current understanding of the nephron. *CJASN* will provide careful reviews of the nephron, sequentially, from the glomerulus to the collecting duct, followed by a review on the control of urinary drainage and bladder function. Next, there will be a series of cohesive reviews that will address how the kidney factors in the integrated response to sodium and water homeostasis, potassium handling, acid-base homeostasis, excretion of organic cations and anions, and divalent cations and phosphate homeostasis. Protein metabolism and control of renal nitrogen excretion as well as sensory functions of the kidney will follow. Finally, the role of the interstitium and hormonal function of the kidney will be considered.

In jingoistic banter that many nephrologists can echo with sincerity, Homer Smith asserted, “The responsibility for maintaining the composition of [the internal milieu] . . . devolves to the kidneys. It is no exaggeration to say that the composition of the body fluids is determined not by what the mouth takes in but by what the kidneys keep; they are the master chemists of our internal environment” (13). With this series in hand, the *CJASN* reader will be privy to the cutting-edge science of nephrology; knowledge of the dramatic advances in our understanding will likely turn any nephrologist into a philosopher.

Authors’ Note

The landmark works described in this article are freely accessible on the Internet for those who would like to indulge in the primary sources. These texts and manuscripts have been made available as part of Google Scholar and the Internet Archive, nonprofit digital libraries with the mission to allow universal access to all knowledge. These archival texts include Homer Smith’s *The Principals of Renal Physiology* and *From Fish to Philosopher*, Bowman’s treatise *On the Structure and Use of the Malpighian Bodies of the Kidney*, and texts by Starling, Cushny, Cannon, and Bernard.

Disclosures

None.

References

1. Smith HW: *Principles of Renal Physiology*, New York, Oxford University Press, 1956

2. Smith HW: The fate of sodium and water in the renal tubules. *Bull N Y Acad Med* 35: 293–316, 1959
3. Bernard C: *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux*, Paris, Baillière JB, 1878
4. Bernard C: *An Introduction to the Study of Experimental Medicine 1865*, London, Macmillan & Co Ltd, 1927
5. Gross CG: Claude Bernard and the constancy of the internal environment. *Neuroscientist* 4: 380–385, 1998
6. Starling EH: The Harvurian Oration, delivered before The Royal College of Physicians of London on St. Luke's Day, 1923. *BMJ* 2: 685–690, 1923
7. Cannon WB: *Wisdom of the Body*, New York, WW Norton, 1939
8. Bowman W: On the structure and use of the malpighian bodies of the kidney, with observations on the circulation through that gland. *Philos Trans R Soc Lond* 132: 57–80, 1942
9. Eknayan G: Sir William Bowman: His contributions to physiology and nephrology. *Kidney Int* 50: 2120–2128, 1996
10. Kinne-Saffran E, Kinne RK: Jacob Henle: The kidney and beyond. *Am J Nephrol* 14: 355–360, 1994
11. Wearn HT, Richards AN: Observations on the composition of glomerular urine, with particular reference to the problem of reabsorption in the renal tubules. *Am J Phys* 71: 209–227, 1924
12. Gottschalk CW: A history of renal physiology to 1950. in: *The Kidney: Physiology and Pathophysiology*, edited by Seldin DW, Giebisch G, 2nd Ed., New York, Raven Press, Ltd, 1992
13. Smith HW: *From Fish to Philosopher*, Garden City, NY, Doubleday and Company, Inc, 1961
14. Cushny AR: The secretion of urine. In: *Monographs on Physiology*, edited by Starling EH, London, Longmans, Green and Co., 1917
15. Sands JM: Micropuncture: Unlocking the secrets of renal function. *Am J Physiol Renal Physiol* 287: F866–F867, 2004
16. Walker AM, Bott PA, Oliver J, MacDowell MD: The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am J Physiol* 134: 580–595, 1941
17. Richards AN, Walker AM: Methods of collecting fluid from known regions of the renal tubules of amphibia and of perfusing the lumen of a single tubule. *Am J Physiol* 118: 111–120, 1937
18. Ussing HH, Zerahn K: Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol Scand* 23: 110–127, 1951
19. Burg MB, Grantham J, Abramow M, Orloff J, Schafer JA: Preparation and study of fragments of single rabbit nephrons. *J Am Soc Nephrol* 8: 675–683, 1997
20. Burg MB, Knepper MA: Single tubule perfusion techniques. *Kidney Int* 30: 166–170, 1986
21. Peitzman SJ: The flame photometer as engine of nephrology: A biography. *Am J Kidney Dis* 56: 379–386, 2010
22. Gamble JL: *Chemical Anatomy, Physiology and Pathology of Extracellular Fluid, A Lecture Syllabus*, Cambridge, Harvard University Press, 1942
23. Harvey AM: Classics in clinical science: James L. Gamble and "Gamblegrams". *Am J Med* 66: 904–906, 1979
24. Hollander W, Chobanian AV, Wilkins RW: Relationship between diuretic and antihypertensive effects of chlorothiazide and mercurial diuretics. *Circulation* 19: 827–838, 1959
25. Chobanian AV, Lille RD, Tercyak A, Blevins P: The metabolic and hemodynamic effects of prolonged bed rest in normal subjects. *Circulation* 49: 551–559, 1974
26. Bonventre JV, Leaf A: Sodium homeostasis: Steady states without a set point. *Kidney Int* 21: 880–883, 1982
27. Skou JC: The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim Biophys Acta* 23: 394–401, 1957
28. Skou JC: Nobel Lecture. The identification of the sodium pump. *Biosci Rep* 18: 155–169, 1998
29. Katz AI: Renal Na-K-ATPase: Its role in tubular sodium and potassium transport. *Am J Physiol* 242: F207–F219, 1982
30. Maddox DA, Deen WM, Brenner BM: Dynamics of glomerular ultrafiltration. VI. Studies in the primate. *Kidney Int* 5: 271–278, 1974
31. Bohrer MP, Baylis C, Humes HD, Glasscock RJ, Robertson CR, Brenner BM: Permeability of the glomerular capillary wall. Facilitated filtration of circulating polycations. *J Clin Invest* 61: 72–78, 1978
32. Drummond IA: Kidney development and disease in the zebrafish. *J Am Soc Nephrol* 16: 299–304, 2005
33. Narayanan S, Appleton HD: Creatinine: A review. *Clin Chem* 26: 1119–1126, 1980
34. Thurau K, Boylan JW: Acute renal success. The unexpected logic of oliguria in acute renal failure. *Am J Med* 61: 308–315, 1976
35. Peti-Peterdi J, Harris RC: Macula densa sensing and signaling mechanisms of renin release. *J Am Soc Nephrol* 21: 1093–1096, 2010
36. Féraille E, Doucet A: Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: Hormonal control. *Physiol Rev* 81: 345–418, 2001
37. Suki W, Rector FC Jr, Seldin DW: The site of action of furosemide and other sulfonamide diuretics in the dog. *J Clin Invest* 44: 1458–1469, 1965
38. Bartter FC, Pronove P, Gill JR Jr, MacCardle RC: Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis. A new syndrome. *Am J Med* 33: 811–828, 1962
39. Proesmans W: Threading through the maze of Bartter syndrome. *Pediatr Nephrol* 21: 896–902, 2006
40. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR Jr, Ulick S, Milora RV, Findling JW, Canessa CM, Rossier BC, Lifton RP: Liddle's syndrome: Heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* 79: 407–414, 1994
41. Zeidel ML, Kikeri D, Silva P, Burrows M, Brenner BM: Atrial natriuretic peptides inhibit conductive sodium uptake by rabbit inner medullary collecting duct cells. *J Clin Invest* 82: 1067–1074, 1988
42. Montani J, Van Vliet BN: Understanding the contribution of Guyton's large circulatory model to long-term control of arterial blood pressure. *Exp Physiol* 94: 382–398, 2009
43. Edelman IS, Leibman J, O'Meara MP, Birkenfeld LW: Interrelations between serum sodium concentration, serum osmolality and total exchangeable sodium, total exchangeable potassium and total body water. *J Clin Invest* 37: 1236–1256, 1958
44. Verney EB: Renal excretion of water and salt. *Lancet* 273: 1237–1242, 1957
45. Knepper MA: Molecular physiology of urinary concentrating mechanism: Regulation of aquaporins water channels by vasopressin. *Am J Physiol Renal Physiol* 272: F3–F12, 1997
46. Nielsen S, Frøkiaer J, Marples D, Kwon TH, Agre P, Knepper MA: Aquaporins in the kidney: From molecules to medicine. *Physiol Rev* 82: 205–244, 2002
47. Pitts RF, Lotspeich WD, Schiess WA, Ayer JL, Miner P: The renal regulation of acid-base balance in man; the nature of the mechanism for acidifying the urine. *J Clin Invest* 27: 48–56, 1948
48. Boron WF: Acid-base transport by the renal proximal tubule. *J Am Soc Nephrol* 17: 2368–2382, 2006
49. Curthoys NP, Gstraunthaler G: Mechanism of increased renal gene expression during metabolic acidosis. *Am J Physiol Renal Physiol* 281: F381–F390, 2001
50. Koeppen BM: The kidney and acid-base regulation. *Adv Physiol Educ* 33: 275–281, 2009
51. Arbus GS, Herbert LA, Levesque PR, Etsten BE, Schwartz WB: Characterization and clinical application of the "significance band" for acute respiratory alkalosis. *N Engl J Med* 280: 117–123, 1969
52. Brackett NC Jr, Wingo CF, Muren O, Solano JT: Acid-base response to chronic hypercapnia in man. *N Engl J Med* 280: 124–130, 1969
53. Krapf R, Pearce D, Lynch C, Xi XP, Reudelhuber TL, Pouyssegur J, Rector FC Jr: Expression of rat renal Na/H antiporter mRNA levels in response to respiratory and metabolic acidosis. *J Clin Invest* 87: 747–751, 1991
54. Berliner RW, Kennedy TJ Jr: Renal tubular secretion of potassium in the normal dog. *J Am Soc Nephrol* 9: 1341–1344, discussion 1344–1345, 1998
55. Malnic G, Klose RM, Giebisch G: Micropuncture study of renal potassium excretion in the rat. *Am J Physiol* 206: 674–686, 1964
56. Giebisch G: Renal potassium transport: Mechanisms and regulation. *Am J Physiol* 274: F817–F833, 1998

57. Greger R, Schlatter E, Hebert SC: Milestones in nephrology: Presence of luminal K⁺, a prerequisite for active NaCl transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. *J Am Soc Nephrol* 12: 1788–1793, 2001
58. Sansom SC, Welling PA: Two channels for one job. *Kidney Int* 72: 529–530, 2007
59. Peacock M: Calcium metabolism in health and disease. *Clin J Am Soc Nephrol* 5[Suppl 1]: S23–S30, 2010
60. Felsenfeld AJ, Levine BS: Milk alkali syndrome and the dynamics of calcium homeostasis. *Clin J Am Soc Nephrol* 1: 641–654, 2006
61. Nijenhuis T, Vallon V, van der Kemp AW, Loffing J, Hoenderop JG, Bindels RJ: Enhanced passive Ca²⁺ reabsorption and reduced Mg²⁺ channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. *J Clin Invest* 115: 1651–1658, 2005
62. Gagnon KB, Delpire E: Physiology of SLC12 transporters: Lessons from inherited human genetic mutations and genetically engineered mouse knockouts. *Am J Physiol Cell Physiol* 304: C693–C714, 2013
63. Hou J, Rajagopal M, Yu ASL: Claudins and the kidney. *Annu Rev Physiol* 75: 479–501, 2013
64. Ferrè S, Hoenderop JG, Bindels RJM: Sensing mechanisms involved in Ca²⁺ and Mg²⁺ homeostasis. *Kidney Int* 82: 1157–1166, 2012
65. Dimke H, Monnens L, Hoenderop JG, Bindels RJ: Evaluation of hypomagnesemia: Lessons from disorders of tubular transport. *Am J Kidney Dis* 62: 377–383, 2013
66. Lederer E, Miyamoto K: Clinical consequences of mutations in sodium phosphate cotransporters. *Clin J Am Soc Nephrol* 7: 1179–1187, 2012
67. Hu MC, Shiizaki K, Kuro-o M, Moe OW: Fibroblast growth factor 23 and Klotho: Physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* 75: 503–533, 2013
68. Scheiss WA, Ayer JL, Lotspeich WD, Pitts RF: The renal regulation of acid-base balance in man. Factors affecting the excretion of titratable acid by the normal human subject. *J Clin Invest* 27: 57–64, 1948
69. Jay V: Richard Bright—physician extraordinaire. *Arch Pathol Lab Med* 124: 1262–1263, 2000
70. Lemley KV, Pauling LS: *Thomas Addis: 1881-1949*, Washington, DC, National Academy of Sciences, 1994
71. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 307: 652–659, 1982

Published online ahead of print. Publication date available at www.cjasn.org.



The Glomerulus: The Sphere of Influence

Martin R. Pollak,* Susan E. Quaggin,[†] Melanie P. Hoenig,* and Lance D. Dworkin^{*}

Abstract

The glomerulus, the filtering unit of the kidney, is a unique bundle of capillaries lined by delicate fenestrated endothelia, a complex mesh of proteins that serve as the glomerular basement membrane and specialized visceral epithelial cells that form the slit diaphragms between interdigitating foot processes. Taken together, this arrangement allows continuous filtration of the plasma volume. The dynamic physical forces that determine the single nephron glomerular filtration are considered. In addition, new insights into the cellular and molecular components of the glomerular tuft and their contribution to glomerular disorders are explored.

Clin J Am Soc Nephrol 9: 1461–1469, 2014. doi: 10.2215/CJN.09400913

The Glomerulus

The glomerulus, the filtering unit of the kidney, is a specialized bundle of capillaries that are uniquely situated between two resistance vessels (Figure 1). These capillaries are each contained within the Bowman's capsule and they are the only capillary beds in the body that are not surrounded by interstitial tissue. Therefore, a unique support structure is needed to maintain flow in these essential capillary units. In fact, all of the major components of the filter itself are unique compared with related structures in other capillary beds. The proximal component layer of the glomerular filter itself is a fenestrated endothelium, characterized by the presence of individual fenestrae on the order of 70–100 nm in diameter. These cells drape the luminal aspect of the capillary and permit filtration. The second layer of the filter, the glomerular basement membrane (GBM), is a complex mesh of extracellular proteins, including type IV collagen, laminins, fibronectins, and proteoglycans. The distal layer of the glomerular filter is composed of the visceral epithelial cells, or podocytes. These remarkable cells help to create the filtration slit diaphragm and serve as support to help sustain the integrity of the free-standing capillary loops. A third cell type, the mesangial cells, also contributes to the integrity of the glomerular tuft and the dynamic nature of filtration. Together, this elegant structure permits the formation of the primary glomerular filtrate that enters a space delimited by the visceral and parietal epithelial cells before modification during transit through the tubule.

Hemodynamic Control of Glomerular Filtration at the Single Nephron Level

Although there is considerable variability, the average human kidney is composed of approximately 1 million individual functioning nephrons, each containing a single glomerulus or filtering unit (1). The GFR for the

entire organism is then the sum of the individual filtration rates of approximately 2 million glomeruli in two kidneys. There can be significant differences in the size and filtration rates of individual glomeruli in different regions of the kidney. For example, juxta-medullary nephrons tend to have a larger intraglomerular volume compared with superficial nephrons (2). Intrarenal blood flow distribution varies in physiologic and pathologic situations and this can also affect total GFR for the organism (3,4). Indeed, medullary blood flow appears to be greatest during a water diuresis and reduced in the setting of antidiuresis (4,5), and also appears to be better preserved in renal hypoperfusion. This discussion focuses on the regulation of filtration at the level of a single, typical glomerulus, by which the precise hemodynamic control of glomerular filtration is best understood. Although the majority of the experimental findings were gleaned from work done in Wistar rats (which have superficial glomeruli, in contrast with most mammals), the principles reviewed here are thought to apply to human glomeruli as well.

The Determinants of Glomerular Ultrafiltration

The transcapillary movement of water across the glomerular capillaries is controlled by the same Starling forces that control fluid movement across all capillary beds. Filtration occurs because there is an imbalance between the mean transcapillary hydraulic pressure gradient (ΔP), which favors filtration, and the mean transcapillary oncotic pressure ($\Delta \pi$), which opposes filtration. (Of note, we use the term *hydraulic*, rather than *hydrostatic*, because the latter refers to pressure in a static liquid, whereas hydraulic pressure refers to pressure of fluid moving through tubes.) The resulting net pressure gradient integrated over the entire filtering portion of a single glomerular capillary network is the mean net ultrafiltration pressure ($\bar{P}_{UF}$). This physiologic condition can be expressed mathematically by Equation 1:

*Beth Israel Deaconess Medical Center, Boston, Massachusetts; [†]Feinberg School of Medicine, Northwestern University, Chicago, Illinois; and ^{*}Brown Medical School, Brown University, Providence, Rhode Island

Correspondence:

Dr. Martin Pollak, Beth Israel Deaconess Medical Center, West Campus/Farr 8, 185 Pilgrim Road, Boston, MA 02215. Email: mpollak@bidmc.harvard.edu

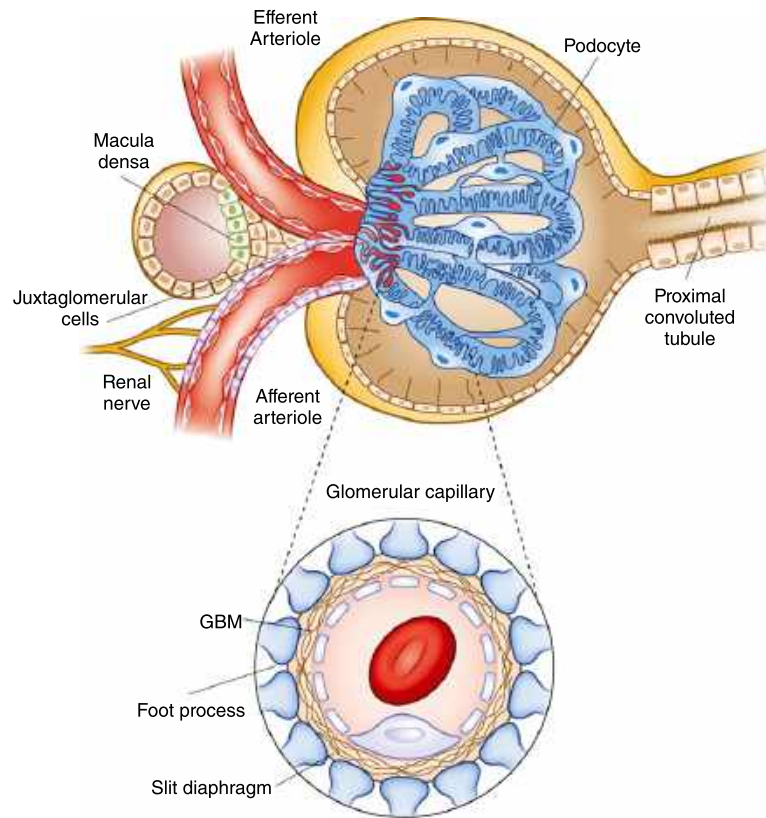


Figure 1. | Structure of the renal corpuscle, looking into the Bowman's capsule at glomerular capillary tuft. The capsule is lined with parietal epithelium, which gives way to the cells of the proximal tubule at the urinary pole on the right. At the left, the vascular pole of the glomerulus includes both the afferent and efferent arterioles. In addition, the relationship between these arterioles and the specialized portion of the distal nephron called the macula densa is illustrated. (Inset) The layers that comprise the filtration barrier are displayed. The outermost layer is composed of the visceral epithelial cells, the podocytes, next the glomerular basement membrane (GBM) and finally the fenestrated endothelial cells.

$$\begin{aligned}
 \text{SNGFR} &= K_f \times (\bar{P}_{UF}) \\
 &= K_f \times (\Delta\bar{P} - \Delta\bar{\pi}) \\
 &= K_f ((\bar{P}_{GC} - P_T) - (\bar{\pi}_{GC} - \pi_T)) \\
 &= k \times S \times ((\bar{P}_{GC} - P_T) - (\bar{\pi}_{GC} - \pi_T)) \quad (1)
 \end{aligned}$$

where SNGFR is the single nephron GFR, $\bar{P}_{GC}$ is the hydraulic pressure within the lumen of the glomerular capillary that is generated by the pumping action of the heart, and P_T is the hydraulic pressure in Bowman's space that is typically measured as the pressure within an early proximal tubule segment. $\bar{\pi}_{GC}$ and π_T are the oncotic pressures within the glomerular capillary, generated by the plasma proteins, and in Bowman's space. Because the sieving characteristics of the glomerular capillary wall that prevent transcapillary filtration of all but small, low molecular weight plasma proteins, π_T is effectively zero. K_f is determined by two factors: the hydraulic conductivity of the glomerular capillary wall, which depends on both its intrinsic characteristics and the concentration polarization of proteins (6) in the capillary (k), and the total capillary surface area available for filtration (S). For any given mean net filtration pressure, $\bar{P}_{UF}$, the absolute amount of filtrate formed will also depend on K_f .

Glomerular Transcapillary Hydraulic and Oncotic Pressures, $\Delta\bar{P}$ and $\Delta\bar{\pi}$. These relationships can also be represented graphically as shown in Figure 2. In this construct, the glomerular capillary network is imagined to be a continuous single tube beginning at the termination of the afferent arteriole, and ending at the origin of the efferent arteriole. The x axis represents fractional distance along the glomerular capillary. Both hydraulic and plasma oncotic pressures are shown on the y axis. The values provided in Figure 2 are typical of those observed in rats, which were first directly measured by Brenner *et al.* (7) and subsequently confirmed in many studies in rats (8) and squirrel monkeys (9). P_{GC} averages just <50 mmHg in normal rats and is nearly constant along the glomerular capillary network, a value significantly higher than in systemic capillaries. The oncotic pressure $\Delta\pi$ rises from approximately 18 to 34 mmHg by the efferent end of the network, a consequence of the filtration process during which water leaves the glomerular capillary lumen causing an increase in protein concentration. π_{GC} equals ΔP by the end of the capillary, resulting in a reduction of local filtration pressure from 17 to 0 mmHg (Figure 2).

The oncotic pressure profile is highly nonlinear and is a consequence of (1) the greater local ultrafiltration pressure near the afferent end of the capillary leading to more rapid translocation of fluid and (2) the exponential relationship

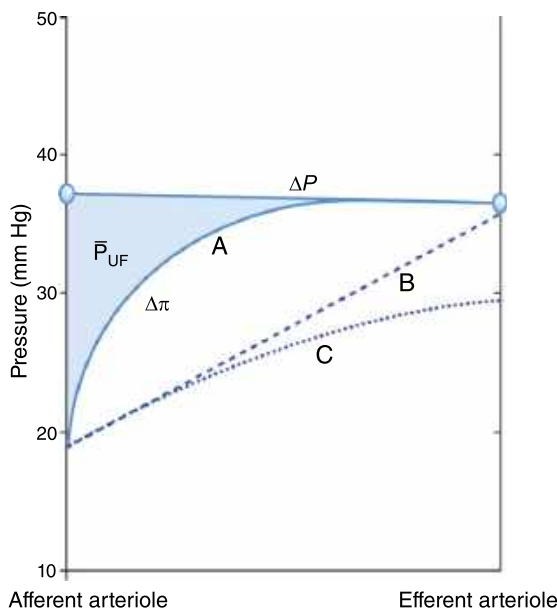


Figure 2. | Hydraulic and oncotic pressures along an idealized glomerular capillary and within Bowman's space. The graph shows idealized hydraulic and oncotic pressure curves under conditions of filtration pressure equilibrium (curve A) when the mean net ultrafiltration pressure ($\bar{P}_{UF}$) is equal to the shaded region between the ΔP and the $\Delta\pi$ curves. Curve B is a hypothetical linear profile of the oncotic pressure that would occur with a minimum value of the K_f . By contrast, in curve C, filtration equilibrium is never reached. Modified from Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASL, Brenner BM: *Brenner and Rector's The Kidney*, 9th Ed., Philadelphia, Elsevier Saunders, 2012, with permission.

between the plasma protein concentration and the plasma oncotic pressure (8). In fact, under equilibrium conditions, the exact $\Delta\pi$ curve cannot be determined. Curve A in Figure 2 is just one of an infinite number of possibilities.

Ultrafiltration Coefficient, K_f . As shown in Equation 1, SNGFR equals the K_f multiplied by the net ultrafiltration pressure integrated over the entire length of the glomerular capillary. The mean net ultrafiltration pressure is represented graphically in Figure 2 as the area between the ΔP and the $\Delta\pi$ curves (shaded blue). As discussed above, because an exact $\Delta\pi$ curve cannot be determined under the normal condition of filtration pressure equilibrium, the exact values for $\bar{P}_{UF}$ and K_f can also not be determined. However, a minimum value for K_f can be estimated using the dashed line in Figure 2 curve B, which gives a maximal possible value for $\bar{P}_{UF}$. Applying this approximation to values for SNGFR and $\Delta\bar{P}$ obtained in rats, K_f has been estimated to range from at least 3.5 to 8 nl/min per mmHg. Using the value of S obtained for rat glomeruli, the calculated value of k is approximately 2500 nl/min per mmHg per cm². This value is one to two orders of magnitude greater than reported for other capillary beds (8), and this allows for the high rate of glomerular filtration despite a net driving pressure of <10 mmHg, on average, along the capillary.

The Effects of Selective Alterations in the Primary Determinants of SNGFR

The four principal determinants of glomerular filtration at the single nephron level are the K_f , the transcapillary

hydraulic pressure difference ($\Delta\bar{P}$), the initial capillary oncotic pressure (π_A), and the initial glomerular plasma flow rate (Q_A) (Figure 3). Selective alteration in any of these four primary determinants has predictable effects on SNGFR, has been examined by mathematical modeling (10) and experimentally (8), and is described here. However, physiologic and pathophysiologic states often engender complex combinations of alterations in multiple determinants that may be additive or offsetting. Indeed, these determinants are not truly independent variables; rather, they tend to have complex and often reciprocal relationships.

Glomerular Capillary Ultrafiltration Coefficient, K_f . Under conditions of filtration pressure equilibrium, which are typically present when Q_A is normal to low, reductions in K_f will not affect SNGFR (Figure 3A). Rather, because less fluid exits the capillary at each point along the network, the oncotic pressure curve is shifted down and to the right, moving the equilibrium point closer to the end of the capillary, as if going from curve A to curve B in Figure 2. This change in the oncotic pressure profile results in an increase in the between the ΔP and $\Delta\pi$, which is equivalent to an increase in the mean net ultrafiltration pressure for the entire capillary network ($\bar{P}_{UF}$). This increase in $\bar{P}_{UF}$ exactly opposes and offsets the decrease in K_f , resulting in constancy in SNGFR. Once K_f is reduced enough to produce disequilibrium, typically by $\geq 50\%$, further reductions in K_f will be associated with declines in SNGFR.

Because K_f is the product of the surface area available for filtration and hydraulic conductivity, reductions in either can affect K_f . Reductions in glomerular surface area may occur in glomerular diseases, such as membranous nephropathy (11) or physiologically, as a result of vasoconstrictors, such as NE (12). Hydraulic conductivity may also decline in glomerular diseases. This has been well characterized in a model for membranous nephropathy (13) and in anti-GBM-induced nephritis (14). In addition, studies in patients with diabetic nephropathy suggest that the hydraulic conductivity is progressively decreased (15). Furthermore, changes in $\Delta\bar{P}$ (16) or a change in the plasma protein concentration (16) (see below) have also been shown to alter hydraulic conductivity.

Transcapillary Hydraulic Pressure Difference, $\Delta\bar{P}$. Until ΔP exceeds π_A , no filtration will occur (Figure 3B). Once the hydraulic pressure exceeds the oncotic pressure, filtration rises with increases in $\Delta\bar{P}$. This relationship is not linear because elevations in $\Delta\bar{P}$ are associated with more fluid filtration earlier in the capillary as well as a more rapid rise in $\Delta\pi$, although not enough to entirely offset the increase in $\Delta\bar{P}$. However, because of the offsetting increase in oncotic pressure and because alterations in $\Delta\bar{P}$ of approximately >10 mmHg are unusual under physiologic conditions (10), changes in $\Delta\bar{P}$ generally result in relatively minor changes in SNGFR.

Oncotic Pressure, π_A . SNGFR is predicted to vary inversely with selective changes in π_A (10) (Figure 3C). This is because increases in oncotic pressure should reduce $\bar{P}_{UF}$ and, thereby, SNGFR. However, changes in π_A do not appear to be an important factor in physiologic regulation of glomerular filtration. First, the plasma protein concentration is relatively stable except in disease states that alter either the production (e.g., in severe liver disease) or degradation or loss of plasma proteins (e.g., in nephrotic syndrome). In addition, changes in plasma protein concentration in mammals are

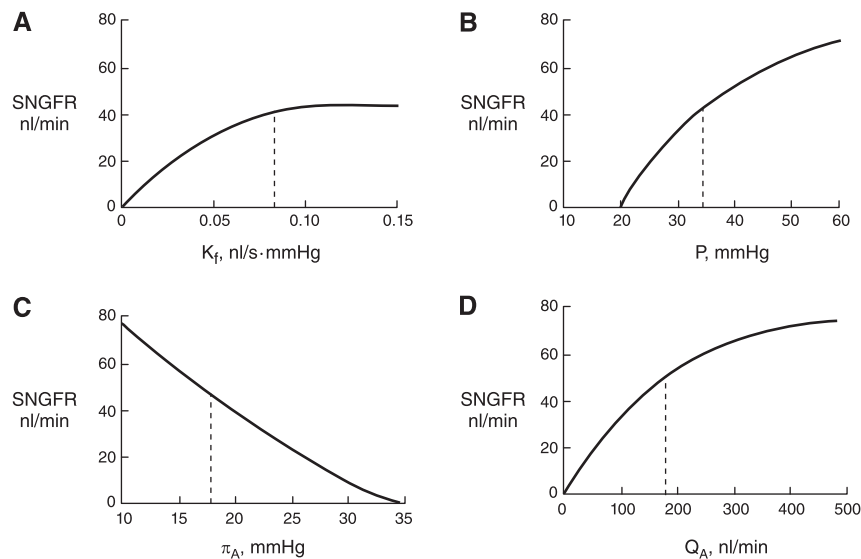


Figure 3. | The single nephron GFR (SNGFR) can be affected by alterations in each of the four major determinants of ultrafiltration. These affects can be expressed in a mathematical model, which helps illustrate the predicted effects of each change on the SNGFR. Normal values are indicated with a dotted line in each graph. Unless otherwise indicated in the graph, the input values are $K_f = 0.095$ nl/s·mmHg, $\Delta\bar{P} = 35$ mmHg, $\pi_A = 18$ mmHg, and $Q_A = 135$ nl/min. (A) Alterations in K_f ; (B) alterations in $\Delta\bar{P}$; (C) alterations in π_A ; (D) alterations in Q_A . Modified from Brenner BM, Dworkin LD, Ichikawa I: Glomerular filtration. In: *Brenner and Rector's The Kidney*, 3rd Ed., edited by Brenner BM, Rector FC Jr, Philadelphia, Elsevier, 1986, with permission.

associated with reciprocal and offsetting alterations in K_f , such that GFR is often unchanged. This is thought to be a result of variation in the hydraulic conductivity of the GBM in response to changes in serum albumin concentration (16), but the exact mechanism is unknown. By contrast, GFR may be reduced in pathologic states in which the oncotic pressure is very high and exceeds hydraulic driving pressure. Case reports of AKI from dextran infusions (17) and massive albumin infusions (18) support this possibility.

Glomerular Plasma Flow Rate, Q_A . Because glomerular filtrate is essentially free of larger plasma proteins, conservation of mass requires that the total amount of protein entering the capillary is equal to the amount leaving it (Figure 3D), which is expressed mathematically in Equation 2:

$$Q_A C_A = Q_E C_E \quad (2)$$

where Q_A and Q_E are the afferent and efferent arteriolar plasma flow rates and C_A and C_E are the respective afferent and efferent arteriolar blood plasma protein concentrations, respectively. Because $Q_E = (Q_A - \text{SNGFR})$, Equation 2 can be rewritten as follows (Equation 3):

$$Q_A C_A = (Q_A - \text{SNGFR}) C_E, \quad (3)$$

which can be rearranged as follows (Equation 4):

$$\text{SNGFR} = (1 - (C_A/C_E)) Q_A \quad (4)$$

Although the relationship between the plasma protein concentration C and the oncotic pressure $\bar{\pi}$ is not linear, Equation 4 is approximately equal to the following (Equation 5):

$$\text{SNGFR} = (1 - (\pi_A/\pi_E)) Q_A \quad (5)$$

Because under conditions of filtration pressure equilibrium, $\pi_E = \Delta\bar{P}$ as follows (Equation 6):

$$\text{SNGFR} = (1 - (\pi_A/\Delta\bar{P})) Q_A \quad (6)$$

Therefore, if π_A and $\Delta\bar{P}$ are held constant, SNGFR will vary directly with Q_A .

This relationship can also be understood graphically by examining Figure 2. As the plasma flow rate increases, there will be less contact time between the plasma flowing through the capillary and any given point along the capillary network. In that case, for any given pressure gradient at any given point, less fluid will leave the capillary as filtrate. As a result, the oncotic pressure profile will be shifted down and to the right, as if moving from curve A to curve B. The equilibrium point is shifted closer to the efferent end of the capillary, resulting in an increase in $\bar{P}_{UF}$ and SNGFR. On the other hand, once Q_A is sufficiently large, disequilibrium will occur, leading to less and less dependence of SNGFR on changes in Q_A . In fact, for animals or under conditions that are far from equilibrium, changes in Q_A are not predicted to have major effects on SNGFR.

Afferent and Efferent Arteriolar Resistances, R_A and R_E

In most physiologic settings, alterations in π_A , K_f , and P_T contribute little to the minute-to-minute regulation of SNGFR. Instead, the filtration rate is regulated by changes in $\bar{P}_{GC}$ and Q_A , and they in turn are controlled by alterations in afferent and efferent arteriolar resistances (R_A and R_E , respectively). The glomerular capillaries are unique in that they are arranged in series between two resistance vessels, the afferent and efferent arterioles. Selective modulation of the relative resistances in these two vessels allows for precise

and largely independent regulation of $\bar{P}_{GC}$ and $\Delta\bar{P}$, Q_A , and SNGFR. In addition, a large number of hormones, vasoactive substances, growth factors, cytokines, and drugs alter resistance in these vessels, selectively or in concert (12).

Renal Autoregulation

One important characteristic of the glomerular circulation is autoregulation, by which not only Q_A but also $\Delta\bar{P}$ and SNGFR are held constant over a wide range of renal artery pressures (Figure 4). Robertson *et al.* examined the precise glomerular hemodynamic mechanisms that account for renal autoregulation (10) by applying micro-puncture techniques to rats. Graded reductions in renal artery pressure were first associated with large declines in R_A , followed by significant increases in R_E . By contrast, an increase in renal plasma flow from vasodilatory substances was associated with a decline in R_E and an increase in Q_A but was offset by a decrease in K_f . These combinations are predicted to be associated with near constancy of $\Delta\bar{P}$, Q_A , and SNGFR, which were indeed observed by these authors for renal artery pressures from approximately 80 to 120 mmHg.

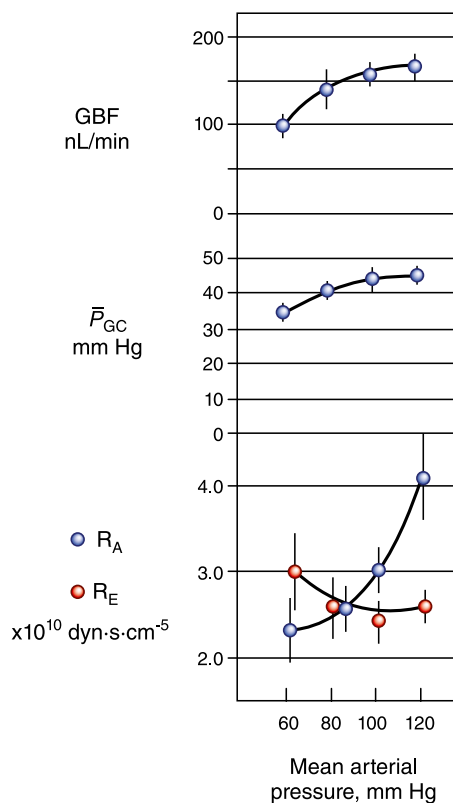


Figure 4. | The effects of graded reduction in renal artery perfusion pressure on glomerular hemodynamics in the rat. Glomerular blood flow (GBF) and glomerular capillary pressure ($\bar{P}_{GC}$) remained relatively constant over the range of pressure from 80 to 120 mmHg, in response to a marked decrease in afferent arteriolar resistance (R_A). With further reduction in perfusion pressure to 60 mmHg, GBF declined proportionally more than $\bar{P}_{GC}$ primarily because of an increase in R_E . Reprinted from Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASL, Brenner BM: *Brenner and Rector's The Kidney*, 9th Ed., Philadelphia, Elsevier Saunders, 2012, with permission.

The mediators responsible for renal autoregulation include a myogenic response that is intrinsic to the blood vessels as well as tubuloglomerular feedback by which chloride uptake by the macula densa segment of the distal tubule is sensed and then elicits effector pathways that modulate R_A and R_E and maintain the SNGFR (3). In addition, changes in the filtration fraction will affect the oncotic pressure in peritubular capillaries and contribute to glomerular tubular balance (19).

Glomerular Filtration of Macromolecules

Classic studies on the separation of the urinary filtrate from the blood highlighted the restriction of serum proteins from the Bowman's space. Detailed *in vivo* studies in both animals and humans have been able to characterize the sieving properties of the glomerular filter, which confers both size and charge selectivity. Studies using dextran molecules of varying sizes and charge demonstrated that neutral particles with a molecular radius >4.2 nm are restricted from the urinary space, whereas anion particles >3.4 nm have a fractional clearance that approaches zero. Thus, the charge of albumin, which is approximately 3.6 nm in size, explains the limited clearance observed (20). By contrast, in both experimental animals and humans with nephrotic syndrome, charge selectivity is lost with the alterations in normal glomerular architecture and this appears to contribute to the protein losses seen in these conditions (21).

Cellular and Molecular Components of the Glomerular Filter

Recent years have seen a rapid increase in knowledge about how the glomerulus and glomerular filtration are regulated at the cellular and molecular levels (Figure 5). Studies of diseases of the three basic components of the glomerular filter have provided a better understanding of the molecular physiology and pathophysiology of this structure. Much of this progress has come through studies of inherited disorders of glomerular function. The glomerular filter is both a charge and size barrier. Movement of anionic molecules across the filter is relatively restricted. Movement of large molecules across the filter is also restricted. The importance of all three components to normal charge and size selectivity is clear from the fact that disruption of any of these three components leads to proteinuric disease states (22).

Disorders of Slit Diaphragm and Podocyte Structure and Function

The glomerular epithelial cell, or podocyte, has been the focus of a great deal of recent investigation. These cells, which are derived from metanephric mesenchyme, form the distal component of the glomerular filter and are both morphologically and functionally unique. Long intricate extensions, or primary processes, lead to secondary or foot processes, which form a complex interdigitating structure with the foot processes from adjacent podocytes. The serpentine cell-cell junction known as the slit diaphragm that bridges these podocytes can be considered a modified adherens junction with some unique additional molecular components (23).

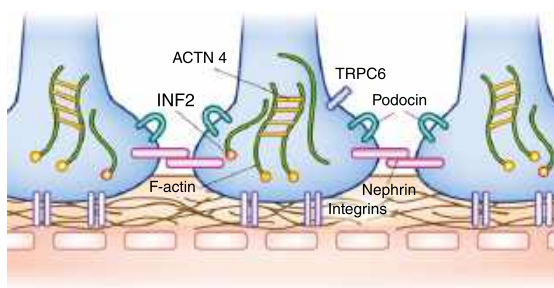


Figure 5. | Major molecular components of the podocyte and slit diaphragm. Interdigitating podocytes from neighboring cells form the elaborate slit diaphragm that is composed of nephrin. Podocin helps regulate trafficking of nephrin to the slit diaphragm. Proteins α -actinin-4 and INF2 play important roles in the maintenance of the actin cytoskeleton, whereas integrins help anchor the podocytes to the glomerular basement membrane. Mutations in α -actinin-4, INF2, and TRPC6 channel all cause autosomal dominant forms of FSGS. Mutations in nephrin and podocin cause recessive forms of steroid-resistant nephrosis.

Alterations in the structure and/or function of the visceral glomerular epithelial cell, or podocyte, lead to a spectrum of human disease. The best established examples are inherited disorders caused by changes in single genes. These inherited “podocytopathies” display a range of phenotypes. As a general rule, recessive forms of podocyte dysfunction are caused by mutations resulting in loss of the function of a normal protein.

The proteins nephrin and podocin are essentially podocyte limited in their expression. Humans, as well as mice, that lack normal copies of either protein develop severe glomerular disease. Both of these molecules were unknown until they were identified on the basis of human genetic studies. Nephrin, a transmembrane domain protein with a large extracellular domain, is required for both normal slit diaphragm structure and function. In Finland, two specific founder mutations in the NPHS1 nephrin gene make this form of congenital nephrotic syndrome much more common than in other parts of the world (24,25). In general, nephrin-mediated congenital nephrotic syndrome manifests as massive proteinuria (≥ 20 g) in the neonatal period. Defects in the NPHS2 podocin gene cause a wider spectrum of disease, ranging from congenital nephrosis to adult-onset FSGS (25,26). Podocin, like nephrin, is an integral membrane protein. Podocin interacts directly with nephrin and appears to help regulate its trafficking to the slit diaphragm (27).

The function of the podocyte *in vivo*, and consequently the function of the entire glomerulus, is very sensitive to mild perturbations in its actin cytoskeleton (28). Mutations in the widely expressed actin-regulating proteins α -actinin-4 and INF2 lead to human forms of podocyte dysfunction, FSGS, and progressive kidney disease, despite having little or no effect on other organ function (29,30). Mutations in the TRPC6 cation channel cause a phenotypically similar autosomal dominant form of FSGS (31).

A good deal of recent work has focused on signaling pathways critical to podocyte function. For example, fine regulation of small GTPase signaling appears essential for maintenance and repair of normal podocyte structure and function (32,33). Autophagy, a catabolic process of cell

“self-eating,” whereby cells degrade unnecessary or dysfunctional intracellular components, is necessary for normal podocyte function. Transgenic mice deficient in autophagy show marked glomerular pathology (34). The communication between slit diaphragm proteins and the cytoskeleton is similarly of critical importance. Proper functioning of the cytoskeletal apparatus is required for organization of the slit diaphragm complex, and normal slit diaphragm function (as both a structural apparatus and signaling center) regulates actin dynamics (34,35).

The functions of some glomerular disease genes and gene products remain unclear. Variants in the APOL1 gene, encoding apoL1, explain the high rate of FSGS and other forms of nondiabetic kidney disease in African Americans. Its function in normal human physiology, if any, is unclear. APOL1 does not exist in rats, mice, and other nonprimates, and is present in only a few primate species.

The GBM

The importance of the GBM to normal glomerular physiology is made evident by the existence of several human diseases characterized by abnormalities in the GBM structure. The GBM is formed by the fusion of basement membranes from both the endothelial and epithelial cells that form the glomerular corpuscle. Morphologically, the GBM contains a dense inner layer called the lamina densa, flanked by the thinner laminae rara interna and rare externa. This morphology, as well as GBM function, is altered in the presence of mutations in type IV collagens, the major components of the GBM. Recessive forms of Alport syndrome can be caused by inheritance of mutations in the autosomal type 4 collagen genes COL3A4 and COL4A4, whereas the more common X-linked form is caused by mutations in COL5A4 (36). Infants born with two mutant copies of the gene encoding the GBM protein laminin β_2 can exhibit congenital nephrotic syndrome (37).

Studies in several mouse models of altered GBM components (type IV collagen, laminin) show that the altered glomerular filters in these models can show increased permeability to large molecules (38). These alterations are seen to occur before ultrastructural abnormalities in the podocyte (39). Thus, altered GBM composition itself can lead to proteinuria. Recent studies have also readdressed the issue of whether glycosaminoglycans in the endothelium help regulate glomerular permeability, confirming the role of these large molecules in charge and size selectivity (40,41).

Despite extensive study, there are still open questions regarding the complex nature of this filter as well as how it allows such high rates of water flow while restricting the flow of large molecules and yet remains functional and “unclogged.” One recent hypothesis suggests that the glomerular filter, and the GBM in particular, acts more like a gel than a simple filter in that the size-selective properties of the glomerular filter are determined by permeation and diffusion properties of the GBM (42).

Endothelium

The glomerular endothelium represents the first layer in the glomerular filtration barrier and is in direct contact with the blood. Although the origin of these cells has been debated for some time, recent lineage tracing studies demonstrated

that they may derive from the Foxd1-positive stromal cell lineage (43). Endothelial cells migrate from the metanephric mesenchyme into the developing glomerular tuft at the S-shape stage of glomerulogenesis. Glomerular endothelial cells are attracted by vascular growth factors, such as vascular endothelial growth factor (VEGF)-A, that are produced by adjacent podocytes (44–46). As glomerular development proceeds, the endothelial cells become progressively flattened and develop fenestrations (two features critical for maintenance of glomerular function), permitting high flux of water and small solutes. The endothelium is covered by a glycocalyx that likely restricts the passage of large macromolecules. Alterations of the glycocalyx were reported in various models of glomerular disease, including diabetic nephropathy (41). Studies in humans have been limited by incomplete knowledge of glycocalyx components and functions and by the need for specialized fixation and staining methods for proper visualization.

Diseases of the Glomerular Endothelium

Primary diseases of the glomerular endothelium may result in rapid loss of kidney function. Thrombotic microangiopathies (TMAs) represent a heterogeneous group of disorders characterized by varying degrees of endothelial swelling known as endotheliosis, fibrin and platelet deposition, splitting of the GBM, and red blood cell fragmentation (47,48). The molecular causes of a number of TMAs were recently elucidated. Although thrombotic thrombocytopenic purpura and hemolytic uremic syndrome (HUS) were once considered to represent a spectrum of the same disease (49), deficiency of ADAMTS13 enzymatic function as a result of congenital mutations or antibody-mediated inhibition is now known to be responsible for thrombotic thrombocytopenic purpura (50). By contrast, HUSs are associated with dysregulation of the alternate pathway of complement. Multiple mutations in genes that encode proteins required for the regulation of complement activation cause atypical HUS (48). Activation of complement leads to perturbation of otherwise thromboresistant renal endothelial cells with resultant local damage and an influx of inflammatory cells. Although the most common form of HUS—diarrheal HUS, caused by Shiga toxin—has not been clearly linked to complement dysregulation, several small series have demonstrated benefit from complement inhibition in patients during a recent large outbreak of diarrheal HUS in Germany (51,52). These data raise the intriguing possibility that complement dysregulation is responsible for renal injury in common forms of HUS as well. Thrombotic injury to the glomerular microvasculature is also a universal feature of the renal disease of preeclampsia and in subsets of patients treated with anti-VEGF agents. Importantly, podocytes produce large amounts of VEGF during development and in filtering adult glomeruli in order to nurture the endothelial cells despite the tide of glomerular filtrate. This paracrine factor appears to be important for maintenance of the normal endothelial cell structure on the basis of observations that inhibition of VEGF signaling within the glomerular endothelium may result in thrombotic injury. Human, mouse, and cell-based studies support this model (53,54). Finally, mutations in the D7E lipid enzyme were identified in families presenting with atypical HUS and/or FSGS (55,56). Endothelial and podocyte injury were observed in biopsies

from these patients, suggesting that phosphorylation of lipid substrates is crucial for glomerular health.

In addition to TMAs, activation of the glomerular endothelium can result in pathologic angiogenesis, enhanced permeability and proteinuria, and upregulation of cell adhesion molecules involved in recruitment of inflammatory cells. These processes are documented in a large number of common glomerular diseases, including diabetic nephropathy and vasculitides. Future studies are needed to determine the molecular basis of endothelial dysfunction, which should provide interesting new therapeutic targets for glomerular disease.

Mesangium

The mesangium refers to the mesangial cells and the matrix they produce. During glomerular development, mesangial cells migrate into the developing tuft under the influence of PDGF β produced by the glomerular endothelial cells acting on the PDGF β receptor expressed by mesangial cells (57). Traditionally, mesangial cells were referred to as pericytes because they provide support to the adjacent capillary loops. However, podocytes also provide essential growth factors and support to the endothelium and they also function as specialized perivascular cells, making the glomerular microvasculature somewhat unique because it has intimate associations with two different supporting cell types. The mesangial processes are filled with bundles of actin and myosin-based microfilaments that extend to contact the GBM, in which they bind laminin $\alpha 5$ *via* integrin $\alpha 3 \beta 1$ and the basal cell adhesion molecule (58). These processes provide protection against glomerular pressure and may regulate glomerular capillary flow *via* contractile properties (59).

The mesangial matrix produced by mesangial cells is composed of a diverse group of proteins, including type III–VI collagens, heparin sulfate proteoglycans, and elastic fiber proteins including fibronectin, laminin, entactin, and fibrillin-1. Accumulation of the mesangial matrix and thickening of the GBM are features commonly observed in a number of glomerular diseases, including diabetic nephropathy. Under these settings, the matrix may include normal as well as new components such as collagen I. Proliferative glomerulonephritides may include mesangial cell proliferation and mesangial deposits.

Newer Methodologies

Much of the “classical” physiology of the mammalian glomerulus has been determined through studies in rats, particularly the extensive studies that have defined the control of filtration at the single nephron level. New experimental tools are expanding the ability to examine glomerular processes. These tools include new animal models, more sophisticated cellular models, and better methods to visualize glomerular function in existing rodent models. For example, multiphoton fluorescence microscopy enables examination of glomerular function *in vivo*, and is now being applied in analyses of models of disease (60). Such studies have confirmed that only a small amount of large proteins is filtered by the glomerulus.

Genetic manipulation of other model organisms, including mice and zebrafish, permits *in vivo* assessment of gene function. Using morpholino technology, it is possible to

rapidly generate “gene knockdowns” in fish and analyze effects on the pronephros, which contains a single glomerulus. More precise modulation of the genome is possible with the multitude of cell and temporally controlled gene knockout strategies available in mice, and development of newer technologies, such as phenotype-driven *N*-ethyl-*N*-nitrosourea (ENU) screens, Clustered Regularly Interspaced Short Palindromic Repeats technology, and short hairpin RNA (shRNA) knockdowns. ENU screening is a technique in which the ENU alkylating is used to induce mutations and then the phenotype of the progeny can be studied and candidate genes from phenotypes of interest can be identified. Clustered Regularly Interspaced Short Palindromic Repeats technology can be used for gene “editing” to assist in the study of specific genetic targets. Finally, shRNA knockdown is a technique that utilizes shRNA sequences to silence a target gene of interest. Combining cell-based studies, model systems, and advances in “omics” will translate into tremendous growth in our knowledge about the physiology and regulation of the glomerulus.

Disclosures

None.

References

- Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van Velzen D: Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. *Lab Invest* 64: 777–784, 1991
- Horster M, Thürau K: Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Pflügers Arch Gesamte Physiol Menschen Tiere* 301: 162–181, 1968
- Gong R, Dworkin LD, Brenner BM, Maddox DA: The renal circulations and glomerular ultrafiltration. In: *Brenner & Rector's The Kidney*, edited by Brenner BM, Philadelphia, Elsevier Saunders, 2008, pp 91–129
- Stein JH, Boonjarern S, Wilson CB, Ferris TF: Alterations in intrarenal blood flow distribution. Methods of measurement and relationship to sodium balance. *Circ Res* 32[Suppl 1]: 61–72, 1973
- Blantz RC, Wallin JD, Rector FC Jr, Seldin DW: Effect of variation in dietary NaCl intake on the intrarenal distribution of plasma flow in the rat. *J Clin Invest* 51: 2790–2795, 1972
- Deen WM, Robertson CR, Brenner BM: Concentration polarization in an ultrafiltering capillary. *Biophys J* 14: 412–431, 1974
- Brenner BM, Troy JL, Daugharty TM: The dynamics of glomerular ultrafiltration in the rat. *J Clin Invest* 50: 1776–1780, 1971
- Maddox DA, Deen WM, Brenner BM: Glomerular Filtration. In: *Handbook of Physiology Renal Physiology*, edited by Windhager EE, Giebisch G, Baltimore, Williams and Wilkins, 1992
- Maddox DA, Deen WM, Brenner BM: Dynamics of glomerular ultrafiltration. VI. Studies in the primate. *Kidney Int* 5: 271–278, 1974
- Deen WM, Robertson CR, Brenner BM: A model of glomerular ultrafiltration in the rat. *Am J Physiol* 223: 1178–1183, 1972
- Squarier A, Lemley KV, Ambalavanan S, Kristal B, Deen WM, Sibley R, Anderson L, Myers BD: Mechanisms of progressive glomerular injury in membranous nephropathy. *J Am Soc Nephrol* 9: 1389–1398, 1998
- Dworkin LD, Ichikawa I, Brenner BM: Hormonal modulation of glomerular function. *Am J Physiol* 244: F95–F104, 1983
- Hladunewich MA, Lemley KV, Blouch KL, Myers BD: Determinants of GFR depression in early membranous nephropathy. *Am J Physiol Renal Physiol* 284: F1014–F1022, 2003
- Blantz RC, Wilson CB: Acute effects of antglomerular basement membrane antibody on the process of glomerular filtration in the rat. *J Clin Invest* 58: 899–911, 1976
- Tomlanovich S, Deen WM, Jones HW 3rd, Schwartz HC, Myers BD: Functional nature of glomerular injury in progressive diabetic glomerulopathy. *Diabetes* 36: 556–565, 1987
- Daniels BS, Hauser EB, Deen WM, Hostetter TH: Glomerular basement membrane: In vitro studies of water and protein permeability. *Am J Physiol* 262: F919–F926, 1992
- Moran M, Kapsner C: Acute renal failure associated with elevated plasma oncotic pressure. *N Engl J Med* 317: 150–153, 1987
- Rozich JD, Paul RV: Acute renal failure precipitated by elevated colloid osmotic pressure. *Am J Med* 87: 359–360, 1989
- Thomson SC, Blantz RC: Glomerulotubular balance, tubuloglomerular feedback, and salt homeostasis. *J Am Soc Nephrol* 19: 2272–2275, 2008
- Brenner BM, Hostetter TH, Humes HD: Molecular basis of proteinuria of glomerular origin. *N Engl J Med* 298: 826–833, 1978
- Guasch A, Deen WM, Myers BD: Charge selectivity of the glomerular filtration barrier in healthy and nephrotic humans. *J Clin Invest* 92: 2274–2282, 1993
- Haraldsson B, Nyström J, Deen WM: Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev* 88: 451–487, 2008
- Reiser J, Kriz W, Kretzler M, Mundel P: The glomerular slit diaphragm is a modified adherens junction. *J Am Soc Nephrol* 11: 1–8, 2000
- Kestilä M, Lenkkeri U, Männikkö M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998
- Tryggvason K, Patrakka J, Wartiovaara J: Hereditary proteinuria syndromes and mechanisms of proteinuria. *N Engl J Med* 354: 1387–1401, 2006
- Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Naudet P, Antignac C: NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000
- Nishibori Y, Liu L, Hosoyamada M, Endou H, Kudo A, Takenaka H, Higashihara E, Bessho F, Takahashi S, Kershaw D, Ruotsalainen V, Tryggvason K, Khoshnoodi J, Yan K: Disease-causing missense mutations in NPHS2 gene alter normal nephrin trafficking to the plasma membrane. *Kidney Int* 66: 1755–1765, 2004
- Faul C, Asanuma K, Yanagida-Asanuma E, Kim K, Mundel P: Actin up: Regulation of podocyte structure and function by components of the actin cytoskeleton. *Trends Cell Biol* 17: 428–437, 2007
- Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodríguez-Pérez JC, Allen PG, Beggs AH, Pollak MR: Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 24: 251–256, 2000
- Brown EJ, Schlondorff JS, Becker DJ, Tsukaguchi H, Tonna SJ, Uscinski AL, Higgs HN, Henderson JM, Pollak MR: Mutations in the formin gene INF2 cause focal segmental glomerulosclerosis. *Nat Genet* 42: 72–76, 2010
- Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL, Pericak-Vance MA, Howell DN, Vance JM, Rosenberg PB: A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science* 308: 1801–1804, 2005
- Greka A, Mundel P: Cell biology and pathology of podocytes. *Annu Rev Physiol* 74: 299–323, 2012
- Mouawad F, Tsui H, Takano T: Role of Rho-GTPases and their regulatory proteins in glomerular podocyte function. *Can J Physiol Pharmacol* 91: 773–782, 2013
- Huber TB, Benzing T: The slit diaphragm: A signaling platform to regulate podocyte function. *Curr Opin Nephrol Hypertens* 14: 211–216, 2005
- George B, Holzman LB: Signaling from the podocyte intercellular junction to the actin cytoskeleton. *Semin Nephrol* 32: 307–318, 2012
- Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG: Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N Engl J Med* 348: 2543–2556, 2003
- Zenker M, Aigner T, Wendler O, Tralau T, Müntefering H, Fenski R, Pitz S, Schumacher V, Royer-Pokora B, Wühl E, Cochat P, Bouvier R, Kraus C, Mark K, Madlon H, Dötsch J, Rascher W,

- Maruniak-Chudek I, Lennert T, Neumann LM, Reis A: Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet* 13: 2625–2632, 2004
38. Suh JH, Miner JH: The glomerular basement membrane as a barrier to albumin. *Nat Rev Nephrol* 9: 470–477, 2013
 39. Jarad G, Cunningham J, Shaw AS, Miner JH: Proteinuria precedes podocyte abnormalities in *Lamb2*^{-/-} mice, implicating the glomerular basement membrane as an albumin barrier. *J Clin Invest* 116: 2272–2279, 2006
 40. Fridén V, Oveland E, Tenstad O, Ebefors K, Nyström J, Nilsson UA, Haraldsson B: The glomerular endothelial cell coat is essential for glomerular filtration. *Kidney Int* 79: 1322–1330, 2011
 41. Jeansson M, Haraldsson B: Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. *Am J Physiol Renal Physiol* 290: F111–F116, 2006
 42. Smithies O: Why the kidney glomerulus does not clog: A gel permeation/diffusion hypothesis of renal function. *Proc Natl Acad Sci U S A* 100: 4108–4113, 2003
 43. Sims-Lucas S, Schaefer C, Bushnell D, Ho J, Logar A, Prochownik E, Gittes G, Bates CM: Endothelial progenitors exist within the kidney and lung mesenchyme. *PLoS ONE* 8: e65993, 2013
 44. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111: 707–716, 2003
 45. Eremina V, Cui S, Gerber H, Ferrara N, Haigh J, Nagy A, Ema M, Rossant J, Jothy S, Miner JH, Quaggin SE: Vascular endothelial growth factor a signaling in the podocyte-endothelial compartment is required for mesangial cell migration and survival. *J Am Soc Nephrol* 17: 724–735, 2006
 46. Eremina V, Baelde HJ, Quaggin SE: Role of the VEGF—a signaling pathway in the glomerulus: Evidence for crosstalk between components of the glomerular filtration barrier. *Nephron, Physiol* 106: 32–37, 2007
 47. Coppo P, Veyradier A: Thrombotic microangiopathies: Towards a pathophysiology-based classification. *Cardiovasc Hematol Disord Drug Targets* 9: 36–50, 2009
 48. Noris M, Remuzzi G: Atypical hemolytic-uremic syndrome. *N Engl J Med* 361: 1676–1687, 2009
 49. Remuzzi G: HUS and TTP: Variable expression of a single entity. *Kidney Int* 32: 292–308, 1987
 50. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, Yang AY, Siemieniak DR, Stark KR, Gruppo R, Sarode R, Shurin SB, Chandrasekaran V, Stabler SP, Sabio H, Bouhassira EE, Upshaw JD Jr, Ginsburg D, Tsai HM: Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 413: 488–494, 2001
 51. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, Boppel T, Kirschfink M, Oualha M, Proulx F, Clermont MJ, Le Deist F, Niaudet P, Schaefer F: Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 364: 2561–2563, 2011
 52. Delmas Y, Vendrely B, Clouzeau B, Bachir H, Bui HN, Lacraz A, Helou S, Bordes C, Reffet A, Llanas B, Skopinski S, Rolland P, Gruson D, Combe C: Outbreak of *Escherichia coli* O104:H4 haemolytic uraemic syndrome in France: Outcome with eculizumab [published online ahead of print November 28, 2013]. *Nephrol Dial Transplant* doi: 10.1093/ndt/gft470
 53. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 111: 649–658, 2003
 54. Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE: VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* 358: 1129–1136, 2008
 55. Lemaire M, Frémeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, Fakhouri F, Taqae S, Nobili F, Martinez F, Ji W, Overton JD, Mane SM, Nürnberg G, Altmüller J, Thiele H, Morin D, Deschenes G, Baudouin V, Llanas B, Collard L, Majid MA, Simkova E, Nürnberg P, Rioux-Leclerc N, Moeckel GW, Gubler MC, Hwa J, Loirat C, Lifton RP: Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet* 45: 531–536, 2013
 56. Ozaltin F, Li B, Rauhauser A, An SW, Soylemezoglu O, Gonul II, Taskiran EZ, Ibsirlioglu T, Korkmaz E, Bilginer Y, Duzova A, Ozen S, Topaloglu R, Besbas N, Ashraf S, Du Y, Liang C, Chen P, Lu D, Vadnagara K, Arbuckle S, Lewis D, Wakeland B, Quigg RJ, Ransom RF, Wakeland EK, Topham MK, Bazan NG, Mohan C, Hildebrandt F, Bakaloglu A, Huang CL, Attanasio M: DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. *J Am Soc Nephrol* 24: 377–384, 2013
 57. Bjarnegård M, Enge M, Norlin J, Gustafsdottir S, Fredriksson S, Abramsson A, Takemoto M, Gustafsson E, Fässler R, Betsholtz C: Endothelium-specific ablation of PDGFB leads to pericyte loss and glomerular, cardiac and placental abnormalities. *Development* 131: 1847–1857, 2004
 58. Kerjaschki D, Ojha PP, Susani M, Horvat R, Binder S, Hovorka A, Hillemanns P, Pytela R: A beta 1-integrin receptor for fibronectin in human kidney glomeruli. *Am J Pathol* 134: 481–489, 1989
 59. Ghayur MN, Krepsinsky JC, Janssen LJ: Contractility of the renal glomerulus and mesangial cells: Lingering doubts and strategies for the future. *Med Hypotheses Res* 4: 1–9, 2008
 60. Peti-Peterdi J, Sipos A: A high-powered view of the filtration barrier. *J Am Soc Nephrol* 21: 1835–1841, 2010



Proximal Tubule Function and Response to Acidosis

Norman P. Curthoys* and Orson W. Moe†

Summary

The human kidneys produce approximately 160–170 L of ultrafiltrate per day. The proximal tubule contributes to fluid, electrolyte, and nutrient homeostasis by reabsorbing approximately 60%–70% of the water and NaCl, a greater proportion of the NaHCO_3 , and nearly all of the nutrients in the ultrafiltrate. The proximal tubule is also the site of active solute secretion, hormone production, and many of the metabolic functions of the kidney. This review discusses the transport of NaCl, NaHCO_3 , glucose, amino acids, and two clinically important anions, citrate and phosphate. NaCl and the accompanying water are reabsorbed in an isotonic fashion. The energy that drives this process is generated largely by the basolateral $\text{Na}^+/\text{K}^+ \text{--ATPase}$, which creates an inward negative membrane potential and Na^+ -gradient. Various Na^+ -dependent countertransporters and cotransporters use the energy of this gradient to promote the uptake of HCO_3^- and various solutes, respectively. A Na^+ -dependent cotransporter mediates the movement of HCO_3^- across the basolateral membrane, whereas various Na^+ -independent passive transporters accomplish the export of various other solutes. To illustrate its homeostatic feat, the proximal tubule alters its metabolism and transport properties in response to metabolic acidosis. The uptake and catabolism of glutamine and citrate are increased during acidosis, whereas the recovery of phosphate from the ultrafiltrate is decreased. The increased catabolism of glutamine results in increased ammoniogenesis and gluconeogenesis. Excretion of the resulting ammonium ions facilitates the excretion of acid, whereas the combined pathways accomplish the net production of HCO_3^- ions that are added to the plasma to partially restore acid-base balance.

Clin J Am Soc Nephrol 9: 1627–1638, 2014. doi: 10.2215/CJN.10391012

Introduction

The extracellular fluid (ECF) space provides a constant environment for the cells of a multicellular organism and prevents wide fluctuations in the ambient environment. This enables the cells to devote their gene products to more productive functions. The kidney is the principal organ that maintains the amount and composition of the ECF by executing functions of excretion, metabolism, and provision of endocrine substances. Most of these kidney functions occur in the proximal tubule, which is an ancient segment in mammalian nephron evolution.

In terms of excretion, the proximal tubule maintains an array of secretory mechanisms inherited from the more archaic secretory nephrons, which are ancestors of mammalian nephrons. The proximal tubule is also a *tour de force* of reabsorption of the glomerular filtrate. The filtration-reabsorption scheme is critical because, as metabolic rates escalated during mammalian evolution, GFR had to increase accordingly. The high GFR mandates a corresponding increase in reabsorption to prevent loss of valuable solutes and water. The proximal tubule fulfills most of the reabsorptive role for NaCl and NaHCO_3 , leaving the fine-tuning to the distal nephron. The proximal tubule also completes the reabsorption of glucose, amino acids, and important anions, including phosphate and citrate, because it is the sole site of transport of these filtered solutes.

In addition to solute reabsorption and secretion, the proximal tubule is also a metabolic organ. For example, within the proximal tubule, 25-hydroxy-vitamin D is converted to 1,25-dihydroxy-vitamin D, a hormone that increases blood Ca^{2+} levels. The proximal tubule is also the site of the 24-hydroxylase reaction that converts 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D to their inactive forms (1). In addition, the proximal tubule is an important site of gluconeogenesis that parallels the liver (2). As an endocrine organ, the kidney also releases erythropoietin, renin, and Klotho into the systemic circulation and produces a plethora of locally active paracrine/autocrine and intracrine hormones, such as dopamine, endothelin, PGs, renin, angiotensin II, and so forth (1,3–5).

Space constraints do not permit a comprehensive account of proximal tubule function in this article. Thus, we will highlight NaCl and NaHCO_3 handling as examples of reclamation of filtrate that are critical in preventing shock and fatal acidosis and where the proximal tubule accomplishes the bulk uptake, leaving the completion to the distal nephron. We will also briefly cover the reabsorption of glucose, amino acids, phosphate, and one organic anion, citrate, where the entire regulatory and absorptive function is confined to the proximal tubule. Whereas glucose and phosphate are primarily returned to the

*Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado; and †Departments of Internal Medicine and Physiology, University of Texas Southwestern Medical Center, Dallas, Texas

Correspondence:

Dr. Norman P. Curthoys, Department of Biochemistry and Molecular Biology, Colorado State University, Campus Delivery 1870, Fort Collins, CO 80523-1870; or Dr. Orson W. Moe, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390. Email: Norman.Curthoys@Colostate.edu or orson.moe@utsouthwestern.edu

circulation, citrate represents one substrate that is partially metabolized in the proximal tubule. Another organic substrate that is absorbed and metabolized is the amino acid glutamine. This process provides the nitrogen and carbon skeleton necessary to support renal gluconeogenesis and ammoniogenesis. Finally, the proximal tubule constantly adjusts its functions in response to needs, which is the hallmark of a stringent homeostatic system (6). Metabolic acidosis represents a state where there is concerted adaptations in multiple proximal tubule transport and metabolic functions aimed at minimization of the effect of the excess acid on the organism and rectification of the disturbance.

NaCl and NaHCO₃ Transport

Na⁺ is the primary cation that maintains the ECF volume (ECFV). Because Cl⁻ is four times more abundant than HCO₃⁻ as an ECFV anion, NaCl balance has become synonymous with ECFV regulation. NaHCO₃ is also a major ECFV solute, second only to NaCl, but it is the principal intracellular and extracellular buffer for H⁺. Thus, NaHCO₃ is better known for its role in acid-base balance than ECFV maintenance. There is limited regulation of gastrointestinal Na⁺, Cl⁻, or HCO₃⁻ absorption so the kidney is the primary organ that regulates external electrolyte balance. The high GFR in humans (160–170 L/d) mandates reabsorption of the valuable filtered solutes. Otherwise, approximately 24,000 mmol of filtered Na⁺ and approximately 4000 mmol of filtered HCO₃⁻ would be lost per day

with disastrous consequences. The proximal tubule is the first nephron segment after the glomerulus where reabsorption commences. It is important to note that proximal solute and water reabsorption proceeds primarily in an isotonic fashion with very small changes in luminal osmolarity. Figure 1A shows the profile of changes in selected solutes along the length of the proximal tubule. Figure 1B shows a generic cell model of how transepithelial transport is achieved. Transporters can broadly be viewed from a thermodynamic standpoint as being driven primarily by changes in enthalpy or entropy. Enthalpy-based or active transporters are directly coupled to ATP hydrolysis. They use energy released from the hydrolysis of phosphoanhydride bonds to move solutes uphill; hence, such transporters are by nature ATPases. Entropy-based or secondary active transporters dissipate existing electrochemical gradients to move a solute against a concentration gradient. Thus, they use the downhill free energy change of one solute to energize the uphill movement of another solute.

Transepithelial transport can occur *via* the paracellular or transcellular route, both of which are driven by electrochemical forces. The energy for solute movement is derived ultimately from high energy bonds in organic substrates taken up from the blood whose catabolism converts the energy into ATP (Figure 1B). Although there are multiple active transport systems directly coupled to ATP hydrolysis, the basolateral Na⁺-K⁺-ATPase is the principal consumer of ATP in the proximal tubule. It creates a low cellular [Na⁺] and negative voltage, which provides the

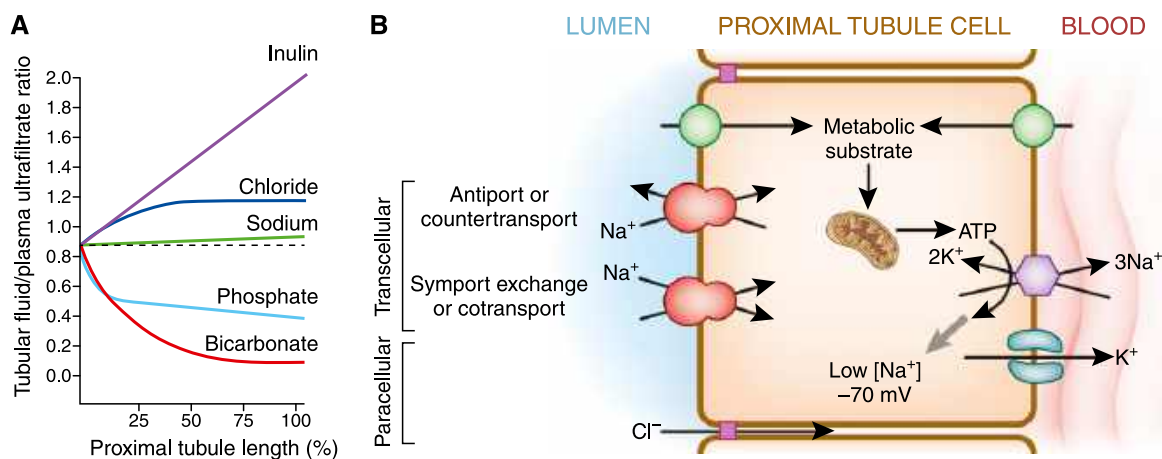


Figure 1. | General considerations of proximal tubule transport. (A) Profile of the tubular fluid to plasma ultrafiltrate ratio (TF/PUF). Selected solutes are shown along the length of the proximal tubule. PUF is a surrogate for the proto-urine in Bowman's space. Inulin represents a filtered molecule that is neither secreted nor reabsorbed and the rise in its TF/PUF solely reflects reabsorption of water, which concentrates luminal inulin. Sodium reabsorption is near isotonic with water, which results in a very small increase in TF/PUF by the end of the proximal tubule. HCO₃⁻ absorption in the early proximal convoluted tubule is particularly avid leading to rapid fall in TF/PUF. The fall in luminal [HCO₃⁻] is accompanied by a reciprocal rise in luminal [Cl⁻] as reabsorption remains by-and-large isotonic. Inorganic phosphate (Pi) reabsorption is more avid in the earlier parts of the proximal tubule. (B) Generic scheme of the proximal tubule cell. The primary energy currency is organic metabolic substrates that enter the proximal tubule and are catabolized to produce ATP, which serves as the secondary energy currency. Some transporters are directly coupled to ATP hydrolysis (enthalpic transport), such as the H⁺-ATPase and Na⁺/K⁺-ATPase. The latter represents the main workhorse of the proximal tubule responsible for the majority of the cellular ATP consumption. The Na⁺/K⁺-ATPase converts the energy stored in ATP into low cellular [Na⁺] and high cellular [K⁺]. The presence of K⁺ conductance allows the [K⁺] gradient to increase the negative interior potential. The low cell [Na⁺] and negative voltage serve as the tertiary energy currencies that drive multiple secondary active apical transporters to achieve uphill movement of solutes coupled to downhill movement of Na⁺ (entropic transport). The transported solutes move in the same (symport or cotransport) or opposite (antiport, exchanger, or countertransport) direction as Na⁺. Movement of solute can also proceed *via* paracellular routes driven by electrochemical forces.

ultimate energy to transport a multitude of solutes across the proximal tubule (Figure 1B). Apical secondary active solute entry can proceed through Na^+ -dependent cotransporters (symporters), exchangers (antiporters), parallel transporter systems, or other Na^+ -independent facilitated transporters (6).

NaCl Transport

Approximately 60%–70% of the filtered NaCl and the accompanying water are reabsorbed by the proximal tubule in a near isotonic fashion. This process is vital to the preservation of ECFV in the face of a high GFR. The foremost driving force is provided by the basolateral Na^+ - K^+ -ATPase, which sets the electrochemical gradient to drive a number of apical transporters that mediate Na^+ and Cl^- entry (Figure 2) as bipartite-coupled parallel exchangers or tripartite-coupled parallel exchangers that all eventuate in net NaCl entry into the cell. Basolateral Cl^- exit is mediated by Cl^- -carrying exchangers and cotransporters (Figure 2) (7–9). Na^+ -coupled transport is a general mechanism in the apical membrane so not all of the Na^+ ions that enter the cell are devoted to NaCl transport (6). For example, a significant amount of glucose enters the apical

membrane *via* Na^+ -glucose cotransport (10). This electrogenic process (net positive charge moving into the cell) contributes to a slight negative luminal potential. In addition, the avid absorption of HCO_3^- in the early proximal tubule and the isotonic nature of the transport elevate the luminal $[\text{Cl}^-]$ to above that of plasma (11). This combined electrochemical driving force in coalition with paracellular Cl^- permeability results in Cl^- movement from urine to blood, which is tantamount to essentially “ Na^+ -glucose- Cl^- absorption” (Figure 2). Alternatively, Na^+ can leak back from the paracellular space into the lumen, providing a recycling system for Na^+ -coupled transport of glucose.

The regulation of proximal tubule NaCl reabsorption facilitates the maintenance of ECFV. Hormones that maintain the ECFV and the integrity of the circulation through antinatriuresis stimulate proximal NaCl absorption. These include angiotensin II (12), endothelin (13), and α -adrenergic stimuli (14). Conversely, natriuretic hormones, such as dopamine, inhibit proximal tubule NaCl reabsorption (15). Hormonal factors regulate largely transcellular NaCl flux *via* modulation of the Na^+ transporters (16). Because NHE3, the apical Na^+/H^+ exchanger, serves both NaCl and NaHCO_3 reabsorption, it is not entirely clear how modulation of these two modes of transport can be dissociated when NHE3 is the prime target of regulation (17). In the proximal tubule, there is also a unique set of regulators that are largely physical in nature and involve coordinate coupling to regulation of GFR. These regulators are unlikely to operate in other nephron segments. When effective arterial blood volume is reduced, GFR is maintained by changes in arteriolar resistances that increase the filtration fraction despite lower renal plasma flow. This increases the protein concentration and oncotic pressure in the postglomerular blood, which along with the lower hydrostatic pressure jointly promote proximal fluid to move into the peritubular capillary.

NaHCO_3 Transport

Whereas NaHCO_3 contributes to the maintenance of ECFV, HCO_3^- is one of the major buffers that protect an organism from constant and pervasive acidification. A 70-kg human contains a free $[\text{H}^+]$ of 40 nM in about 42 L of water. Consumption of a high-protein Western diet results in a net production of 50–70 mEq of H^+ per day. Thus, in the absence of an appropriate buffer, the daily production of H^+ will decrease the body pH to <3 within an hour, which is clearly not compatible with life. HCO_3^- ions provide the major buffer system that prevents the rapid acidification of the ECF. The kidney is the primary organ that controls plasma $[\text{HCO}_3^-]$. The burden of renal transport is to excrete an amount of acid equivalent to the daily net H^+ production plus the amount of filtered HCO_3^- , which is equivalent to the addition of acid if not reclaimed. HCO_3^- reclamation is achieved not so much by HCO_3^- reabsorption but, rather, by H^+ secretion. The approximately 4000 mEq/d of HCO_3^- reclaimed is much higher than any daily dietary acid or base burden. Whether the organism is consuming a net acid diet of 50 mEq H^+ equivalent per day versus a net alkaline diet of 50 mEq OH^- equivalent per day, the H^+ that needs to be secreted into the lumen is 4050 mEq/d versus 3950 mEq/d, respectively. Therefore, the proximal tubular epithelium is

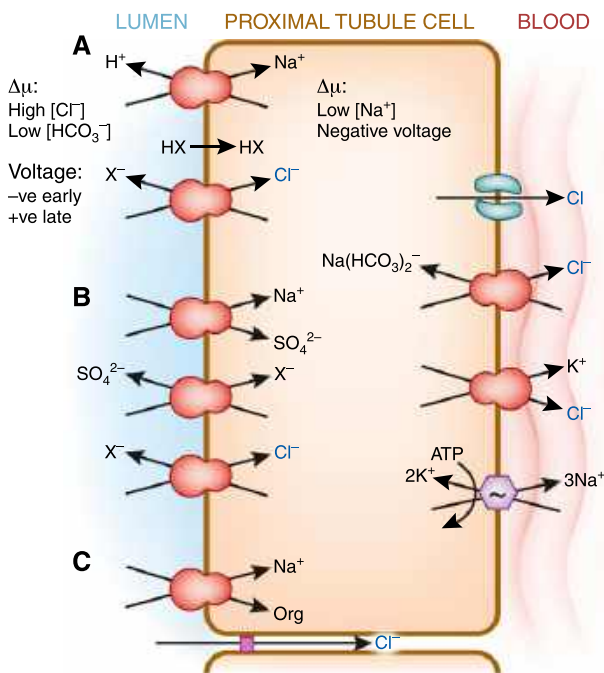


Figure 2. | Proximal tubule NaCl reabsorption. Unlike the thick ascending limb or distal convoluted tubule, there are no dedicated “NaCl” transporters in the proximal tubule. The proximal tubule uses parallel arrays of symporters and antiporters to affect NaCl entry. (A) Parallel Na^+/H^+ exchange (NHE3) and $\text{Cl}^-/\text{base}^-$ exchange (CFEX, SLC26A6) with recycling of the conjugate acid HX resulting in net NaCl entry. (B) A triple coupling mechanism where Na-sulfate cotransport (NaS-1) runs in parallel with two anion exchangers to achieve NaCl cotransport. (C) Na^+ -coupled organic solute transport with concurrent paracellular Cl^- transport driven by the lumen-to-interstitial space downhill chemical gradient of $[\text{Cl}^-]$. Basolateral Na^+ exit is mediated by the Na^+/K^+ -ATPase but some Na^+ also exit with HCO_3^- (see Figure 3). Cl^- exit is less well defined utilizing a variety of transporters and possibly a Cl^- channel.

perpetually engaged in an H⁺-secreting mode regardless of the dietary load.

The proximal tubule plays a pivotal role in many aspects of acid-base homeostasis, which extends beyond HCO_3^- reclamation and H^+ excretion (18). Because the urine cannot possibly maintain a pH low enough to hold 50–70 mEq of free H^+ , urinary buffers carry the majority of the H^+ . The proximal tubule synthesizes the most important open buffer ($\text{NH}_3/\text{NH}_4^+$) and regulates the most abundant closed buffer ($\text{HPO}_4^-/\text{H}_2\text{PO}_4^{2-}$), which will be discussed in more detail below. Finally, the most abundant base equivalent in urine is citrate^{2-/3-}, whose urinary excretion is also exclusively regulated by the proximal tubule (19).

The proximal tubule fulfills the roles of reclamation of HCO_3^- and secretion of H^+ through the uphill transport of H^+ into the lumen, a process collectively referred to as renal acidification. Classic disequilibrium pH experiments have detected very little direct HCO_3^- reabsorption (20). In the proximal tubule, H^+ is secreted into the lumen mostly by electroneutral Na^+/H^+ exchange. The active transport of H^+ by the H^+ -ATPase (V-ATPase) also contributes, but to a lesser extent (Figure 3) (21,22). The main Na^+/H^+ exchanger isoform is NHE3 but NHE8 also participates, particularly in the neonate where NHE3 is not fully expressed (23,24). In addition to H^+ , NHE3 also mediates the secretion of NH_4^+ formed in the cell by titration

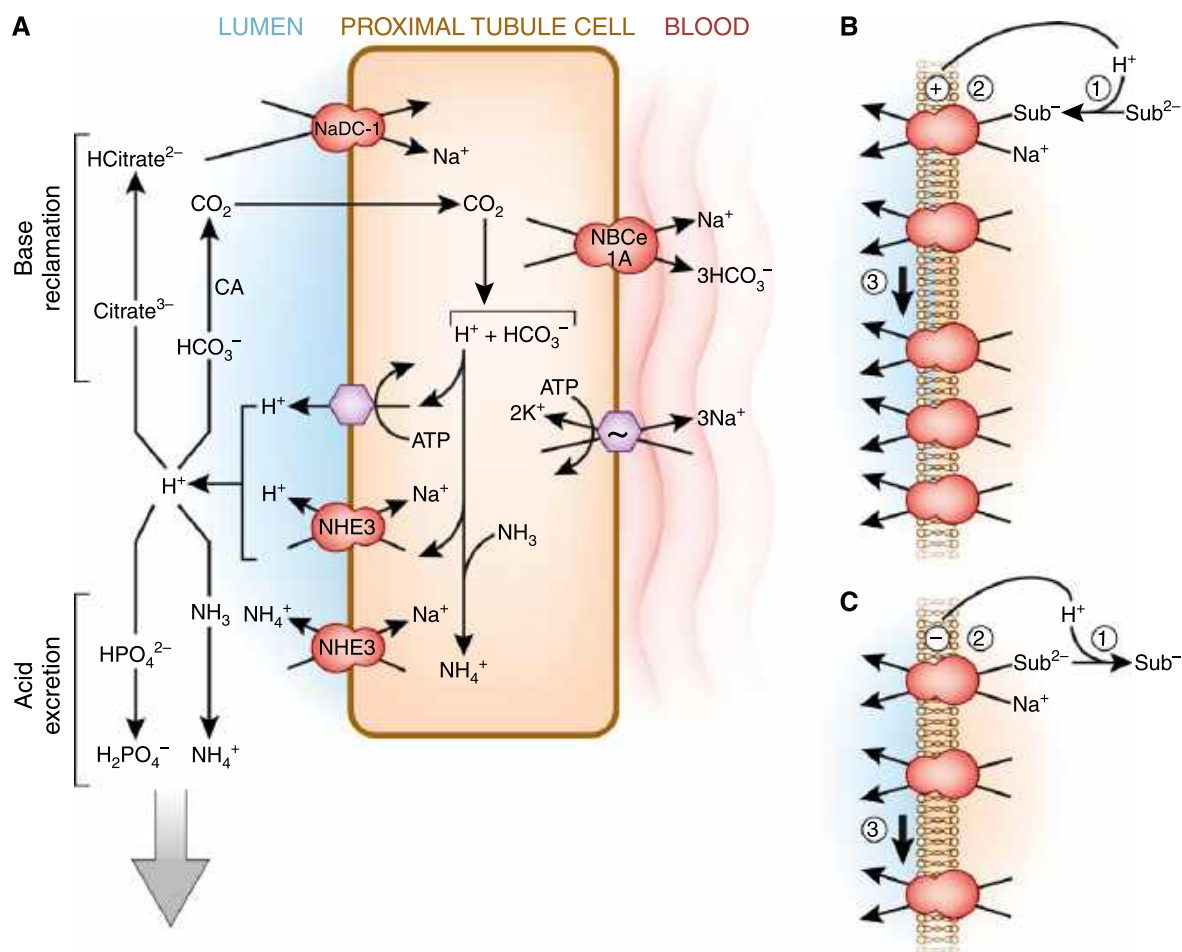


Figure 3. | Proximal tubule HCO_3^- reabsorption and H^+ secretion. Unlike NaCl reabsorption, luminal acidification is mediated by dedicated acid-base transporters. (A) Luminal H^+ secretion is mediated by direct coupling to ATP hydrolysis via the H^+ -ATPase, but a higher proportion of luminal H^+ secretion occurs by the Na^+/H^+ exchanger NHE3. In the neonate before maturational expression of NHE3, NHE8 is the more important NHE isoform. In addition to H^+ , NHE3 also directly transports NH_4^+ formed in the cell from NH_3 and H^+ into the lumen. The H^+ secreted into the lumen has several fates. Reclamation of filtered base is shown on the top left. The titration of filtered HCO_3^- leads to formation of CO_2 under the influence of carbonic anhydrase (CA). The CO_2 enters the cell and is tantamount to HCO_3^- reabsorption. The titration of trivalent citrate to its bivalent form facilitates citrate reabsorption. Citrate is the most important and abundant organic urinary base equivalent. The bottom left depicts acid secretion. In addition to direct $\text{Na}^+/\text{NH}_4^+$ exchange by NHE3, luminal H^+ can combine with NH_3 to form *de novo* NH_4^+ in the lumen. Titration of divalent to monovalent phosphate reduces phosphate absorption and allows the titrated phosphate to function as a H^+ carrier in the urine. CO_2 in the cell is hydrated to H_2CO_3 , which dissociates to form a H^+ and HCO_3^- . The metabolism of citrate²⁻³⁻ also consumes H^+ in the cell, a reaction equivalent to generating HCO_3^- . The generated HCO_3^- exits the cell via Na^+ -coupled transport. There are three generic mechanisms by which changes in luminal and cell pH can alter apical transporters. (B and C) Stimulation is shown in B and inhibition is shown in C. (1) Luminal pH can alter the substrate concentration by titration and regulate transport kinetically. (2) Direct regulatory gating of the transporters by pH. (3) Changes in the number of transporters on the apical membrane by changes in trafficking, protein synthesis, and transcript levels. Sub, substrate.

of NH_3 , which is tantamount to net H^+ secretion (25). The H^+ exported into the lumen has multiple fates (Figure 3A). It combines with the filtered HCO_3^- and, under the action of luminal carbonic anhydrase (CA-IV), generates CO_2 , which diffuses into the cell and reconstitutes H^+ and HCO_3^- . The combined reaction accomplishes the reclamation of filtered HCO_3^- (Figure 3A). The secreted H^+ also titrates citrate from its trivalent form into its divalent form, which is the preferred substrate for uptake by the Na^+ dicarboxylate cotransporter, NaDC-1 (Figure 3B) (26). The reabsorption of citrate^{2- / 3-} is equivalent to reabsorption of alkali (27). Finally, H^+ secretion also titrates divalent HPO_4^{2-} to monovalent H_2PO_4^- that is not transported by NaPi-2a and NaPi-2c (Figure 3B), leading to phosphaturia and increased titratable acid or nonammonia urinary buffer carrying H^+ ions in the urine. The HCO_3^- generated intracellularly by apical H^+ secretion or ammoniogenesis exits the basolateral membrane *via* the family of Na^+ -bicarbonate cotransporters (28). Notably, the highly electrogenic NBCe1A (NBC1, SLC4A4), which is a splice variant of the electrogenic family of NBCe1, is responsible for basolateral HCO_3^- exit (29).

Approximately 70%–90% of the filtered HCO_3^- is reabsorbed by the proximal tubule. Thus, this segment plays a pivotal role in the reclamation of HCO_3^- . In terms of net H^+ excretion, all of the $\text{NH}_3/\text{NH}_4^+$ in the final urine is synthesized in the proximal tubule. The luminal pH at the end of the proximal straight tubule is approximately 6.7–6.8, which means virtually all of the NH_3 is titrated to NH_4^+ ($\text{pK} = 9.3$). Likewise, half of the phosphate is already in its monovalent form. Therefore, the proximal tubule plays an essential role in net acid excretion because a large fraction of the urinary buffers is already titrated by the end of the proximal tubule.

Other Solute Transport

Another primary function of the proximal tubule is the recovery of metabolites from the glomerular filtrate. Approximately 180 g of glucose (1000 mEq) and 50 g of amino acids (400 mEq) are filtered by the human kidney per day. The process of transepithelial transport normally accomplishes the recovery of 99.8% of these metabolites from the luminal fluid of the proximal tubule. As mentioned previously, this process requires the asymmetric association of distinct transporters in the apical and basolateral membranes. Typically, a secondary active Na^+ -dependent transporter in the brush border membrane uses the Na^+ gradient to accomplish the initial uptake of solutes. By contrast, the subsequent transport of the solutes across the basolateral membrane frequently utilizes a Na^+ -independent passive transporter. Important examples of transepithelial transport are those involved in the recovery of glucose, glutamine, citrate, and phosphate.

Glucose Transport

Two distinct Na^+ -dependent transporters mediate the uptake of glucose from the lumen of the proximal tubule (30). SGLT2 (SLC5A2) is a moderate affinity glucose transporter that mediates the cotransport of glucose and one Na^+ ion. The SGLT2 transporter is localized to the brush border membrane of the S1 and S2 segments of the

proximal tubule where it extracts the bulk of the filtered glucose. By contrast, SGLT1 (SLC5A1) has a slightly greater affinity, cotransports two Na^+ ions per glucose, and is preferentially expressed in the apical membrane of the S3 segment. The cotransport of two Na^+ ions makes the uptake of glucose energetically more favorable and thereby increases the concentrative power of the SGLT1. Thus, the sequential positioning of the two transporters that favor capacity and affinity in the early and late tubule, respectively, creates an effective mechanism to ensure that <1% of the filtered glucose exits the proximal tubule. Selective knockout studies indicate that SGLT2 normally accounts for 97% of the net glucose reabsorption, whereas SGLT1 removes the residual glucose and provides reserve capacity (31). Selective inhibition of SGLT2 has been proposed as a potential therapy for treatment of diabetes that may ameliorate glycemia and reduce the associated hyperfiltration (32,33). The basolateral membranes of the early and late segments of the proximal tubule contain the Na^+ -independent glucose transporters, GLUT2 and GLUT1, respectively. Both transporters facilitate the passive movement of glucose from the proximal tubular cells to the interstitial space. The proximal tubule metabolizes little or no glucose, which is compatible with the very low hexokinase in this segment (34). In normal acid-base balance, the arterial-venous difference for glucose across the kidney is zero or slightly positive, which reflects substantial proximal tubule gluconeogenesis counterbalanced by glucose consumption by the rest of the nephron segments (35).

Amino Acid Transport

The renal transport of amino acids is a complex process due to the range of structures and ionic properties of the free amino acids in the plasma (36,37). However, >80% of the filtered amino acids are neutral amino acids, all of which are recovered by the apical $\text{B}^0\text{AT1}$ (SLC6A19) transporter. $\text{B}^0\text{AT1}$ is a broad specificity Na^+ -dependent cotransporter that is expressed in the early portion of the proximal tubule and that binds various neutral amino acids, including glutamine, with relatively low affinities (38). Previous micropuncture studies established that the filtered glutamine is nearly quantitatively reabsorbed from the lumen of the early proximal tubule (39). Mutations in the $\text{B}^0\text{AT1}$ transporter result in Hartnup disorder, which is characterized by a pronounced urinary loss of neutral amino acids (40,41). A separate Na^+ and H^+ -dependent cotransporter (SLC1A1) recovers the acidic amino acids (42), whereas an antiporter (SLC7A9) mediates the uptake of basic amino acids and cysteine in exchange for a neutral amino acid (37). The transporters that mediate the export of amino acids across the basolateral membrane are less well characterized. However, it is thought that in normal acid-base balance, LAT2-4F2hc (SLC7A8), a heterodimeric obligatory neutral amino acid exchanger, mediates the efflux of glutamine from the proximal tubule (36,43). A related heterodimeric exchanger, $\text{y}^+\text{LAT1-4F2hc}$ (SLC7A7), promotes the export of basic amino acids in exchange for a neutral amino acid (44). The two antiporters contribute to the maintenance of normal intracellular levels of amino acids. However, net efflux requires the participation of a uniporter that facilitates the passive export of neutral

amino acids. The TAT1 transporter (SLC16A10) may accomplish the latter process (37).

Organic Cation and Anion Transport

The proximal tubule handles a very broad range of organic cations and anions that utilizes a variety of transporters operating in absorptive and secretory modes (45); however, due to space limitations, we will focus our discussion on citrate. The reabsorption of filtered citrate occurs in the proximal tubule apical membrane by NaDC-1 (SLC13A2), a Na^+ -dependent dicarboxylic acid cotransporter (46). The preferred substrates are dicarboxylates, such as citrate, succinate, fumarate, and α -ketoglutarate. In the proximal tubular lumen, citrate exists in equilibrium between its divalent and trivalent forms but citrate²⁻ is the transported species. Once it is absorbed from the lumen, citrate can be metabolized by cytoplasmic ATP citrate lyase to oxaloacetate and acetyl-CoA or shuttled into the mitochondria where it enters the citric acid cycle (47). When citrate^{2-/3-} is converted to CO_2 and H_2O , 2 or 3 H^+ ions are consumed. Therefore, each milliequivalent of citrate excreted in the urine is tantamount to 2 or 3 OH^- lost. In the normal day-to-day setting in a human on a Western diet, there is a negligible amount of HCO_3^- in the urine. Citrate is the only organic anion in millimolar quantities in the urine and represents the main mode of base excretion under normal circumstances. In the face of large alkali loads, bicarbonaturia becomes the main mechanism of base excretion.

Citrate serves a dual purpose in the urine. As indicated above, it is a urinary base. In addition to base excretion, the 1:1 Ca^{2+} :citrate³⁻ complex has a very high association

constant and solubility. These properties render citrate the most effective chelator of calcium in the urine, which prevents its precipitation with phosphate and oxalate (48,49). Hypocitraturia is a major underlying cause of human kidney stones and, thus, citrate is the most important urinary anion for clinicians to understand (49).

Phosphate Transport

Phosphate homeostasis at the whole-organism level involves coordinated fluxes in the intestine, bone, and kidney, and endocrine cross-talk constituting a complex multiorgan network (50). Although both the intestine and bone are critical organs for phosphate homeostasis, this manuscript will only discuss the renal component. It is important to state that intestinal phosphate absorption involves significant paracellular uptake that is poorly regulated (51). Thus, the kidney is the paramount regulator of external balance. Unlike Na^+ reabsorption where the finishing fine-tunings are achieved by more distal segments, phosphate reabsorption is accomplished almost entirely by the proximal tubule (52). A small contribution from the distal tubule has been proposed, but is still disputed (53). Plasma levels reflect total body phosphate status but are very insensitive; spot urinary concentrations are confounded by water excretion rate; and excretion rates of phosphate are affected by ingestion. Thus, all of these parameters are less than ideal to evaluate renal tubular phosphate handling. The parameters listed in Figure 4A are better suited to probe the proximal tubular handling independent of the excreted or filtered load.

The cellular model for proximal tubule phosphate transport is shown in Figure 4B. The primary driving force is the

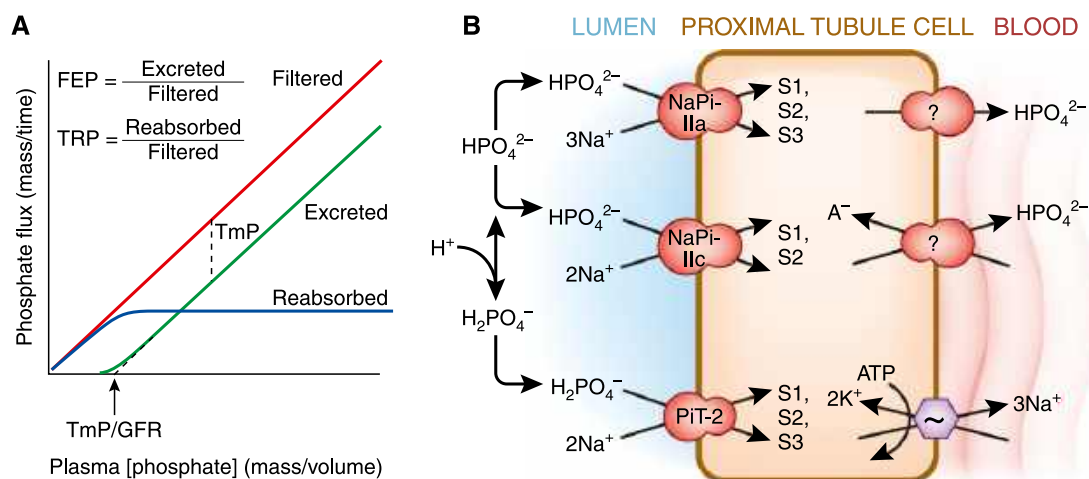


Figure 4. | Proximal tubule phosphate transport. (A) Concepts of renal inorganic phosphate (P_i) homeostasis by the proximal tubule. The flux of filtered and reabsorbed P_i is plotted against plasma phosphate concentration; the difference between the two yields the rate of excretion of P_i . There are a number of terms used to quantify the proximal tubule's P_i reabsorption at the whole organism level. Fractional excretion of P_i (FEP) and tubular reabsorption of P_i (TRP) sum to unity ($\text{FEP} = 1 - \text{TRP}$). The maximal tubular reabsorptive capacity of P_i (TmP in units of mass/time) refers to the saturating transepithelial flux of P_i that the tubule can mount and is equal to the difference between filtered and absorbed phosphate when the filtered load is higher than TmP. The plasma concentration threshold at which P_i starts to appear in the urine is TmP/GFR (in units of mass/volume). (B) Cell model of proximal tubule P_i transport. Three apical transporters mediate P_i entry with different preferred valence of P_i , stoichiometry of Na^+ , electrogenicity, and pH gating. The affinities for Na^+ are all approximately 30–50 mM but are much higher for phosphate (0.1, 0.07, and 0.025 mM for NaPi-IIa, NaPi-IIc, and PiT-2, respectively). Distribution in the proximal tubule segments (S1, S2, S3). Basolateral P_i exit occurs via unknown mechanisms. Apical Na-coupled P_i transport is inhibited in acidosis by alteration in luminal substrate, directly gating of the transporter by pH, and decreased apical NaPi transporters as described in Figure 3B.

$\text{Na}^+\text{-K}^+\text{-ATPase}$ generating an electrochemical gradient for apical phosphate entry. The current model predicts three transporters for apical entry; NaPi-2a (Npt2a, SLC34A1), NaPi-2c (Npt2c, SLC34A2), and Pit-2 (Npt3, SLC20A2, Ram-1). The disparate properties of the three transporters were reviewed in great detail by Virkki and colleagues (54). At present, the basolateral mechanism of phosphate exit still remains enigmatic. It is possible that one or more of the plethora of anion exchange mechanisms may mediate phosphate exit. Thus far, there is little evidence for regulation of basolateral phosphate exit.

Regulation of phosphate transport at the proximal tubule apical membrane is precise because this is the only and final site of determination of extracellular phosphate balance by the kidney. Phosphate uptake is affected by incoming signals, such as parathyroid hormone (55), dopamine (56), fibroblast growth factor-23 (57), and Klotho (58), which inhibit phosphate transport and induce phosphaturia. One of the most potent regulators of phosphaturia is dietary phosphate intake itself, which may involve a variety of hormones, including unknown intestinal enterokines (59), and direct sensing by the proximal tubule (60). This is one of the most important, yet least known, areas in phosphate homeostasis. The modulation of proximal phosphate transport is achieved largely by trafficking of the transporters in and out of the apical membrane (52) with the exception of Klotho, which can directly affect phosphate transport activity (58).

Response to Acidosis

The catabolism of acidic and sulfur-containing amino acids results in the net production of acids. As a result, a high-protein diet leads to a mild chronic metabolic acidosis that is usually well compensated. The common clinical condition of metabolic acidosis is characterized by a more significant decrease in plasma pH and bicarbonate concentration. This disturbance in acid-base balance can be caused by genetic or acquired alterations in metabolism, in renal handling of bicarbonate, and in the excretion of acid. In addition, patients with cachexia, trauma, uremia, ESRD, and HIV infection frequently develop acidosis as a secondary complication that adversely affects their outcome. Chronic acidosis also causes impaired growth, bone loss, muscle wasting, nephrocalcinosis, and urolithiasis. In acute situations with massive acid production that overwhelms the renal capacity, the kidney's response is not relevant; however, in more chronic conditions, renal compensation is crucial.

An essential renal compensatory response to metabolic acidosis is initiated by increased extraction and catabolism of plasma glutamine that occur predominately in the proximal convoluted tubule. The resulting increases in renal ammoniogenesis and transport into the urine accomplish the excretion of acid, whereas the increased bicarbonate synthesis and transport into the blood partially correct the systemic acidosis. These adaptations occur rapidly after acute onset of acidosis and are subsequently sustained by more gradual changes in gene expression.

During normal acid-base balance, the kidneys extract and metabolize very little of the plasma glutamine. Although approximately 20% of the plasma glutamine is filtered, the measured rat renal arterial-venous difference is

<3% of the arterial concentration of glutamine (61), and only 7% of the plasma glutamine is extracted by the human kidneys even after an overnight fast (62). Therefore, renal utilization is significantly less than the fraction of plasma glutamine filtered by the glomeruli. To account for the effective reabsorption of glutamine, either the activity of the mitochondrial glutamine transporter or the glutaminase must be largely inhibited or inactivated *in vivo* during normal acid-base balance.

Acute onset of metabolic acidosis produces rapid changes in the interorgan metabolism of glutamine (63) that support a rapid and pronounced increase in renal catabolism of glutamine. Within 1–3 hours, the arterial plasma glutamine concentration is increased 2-fold (64) due primarily to an increased release of glutamine from muscle (65). Significant renal extraction of glutamine becomes evident as the arterial plasma concentration is increased. Net extraction by the kidney reaches 35% of the plasma glutamine, a level that exceeds the proportion (20%) filtered by the glomeruli. Thus, the normal direction of the basolateral glutamine exchange transporter, LAT2-4F2hc, is reversed in order for the proximal convoluted tubule cells to extract glutamine from both the glomerular filtrate and the venous blood (Figure 5). In addition, the transport of glutamine into the mitochondria may be acutely activated (66). Additional responses include a prompt acidification of the urine that results from translocation, as evidenced in OKP cells (67), and by acute activation of NHE3 (68). This process facilitates the rapid removal of cellular ammonium ions (69) and ensures that the bulk of the ammonium ions generated from the amide and amine nitrogens of glutamine are excreted in the urine. Finally, the cellular concentrations of glutamate and α -ketoglutarate are significantly decreased within the rat renal cortex (70). The latter compounds are products and inhibitors of the glutaminase and glutamate dehydrogenase reactions, respectively. The decrease in concentrations of the two regulatory metabolites may result from a pH-induced activation of α -ketoglutarate dehydrogenase (69). Thus, the acute increase in renal ammoniogenesis may result from a rapid activation of key transport processes, an increased availability of glutamine, and a decrease in product inhibition of the enzymes of ammoniogenesis.

During chronic acidosis, many of the acute responses are reversed and the arterial plasma concentration is decreased to a new steady state that is 70% of normal. However, more than one third of the plasma glutamine is still extracted in a single pass through the kidneys. The increased renal catabolism of glutamine in the proximal convoluted tubule is now sustained by increased expression of genes that encode key transporters and enzymes of glutamine metabolism (Figure 5). A comprehensive survey of the adaptive response of known amino acid transporters in mouse kidney demonstrated that only the basolateral SNAT3 (SLC38A3) transporter exhibits a rapid and pronounced increase during onset of acidosis (71). The SNAT3 transporter has a high affinity for glutamine (72). Under physiologic conditions, it catalyzes a reversible Na^+ -dependent uptake of glutamine that is coupled to the efflux of H^+ ions (73). SNAT3 is normally localized solely to the basolateral membrane of the S3 segment of the proximal tubule (74). This segment expresses high levels of glutamine

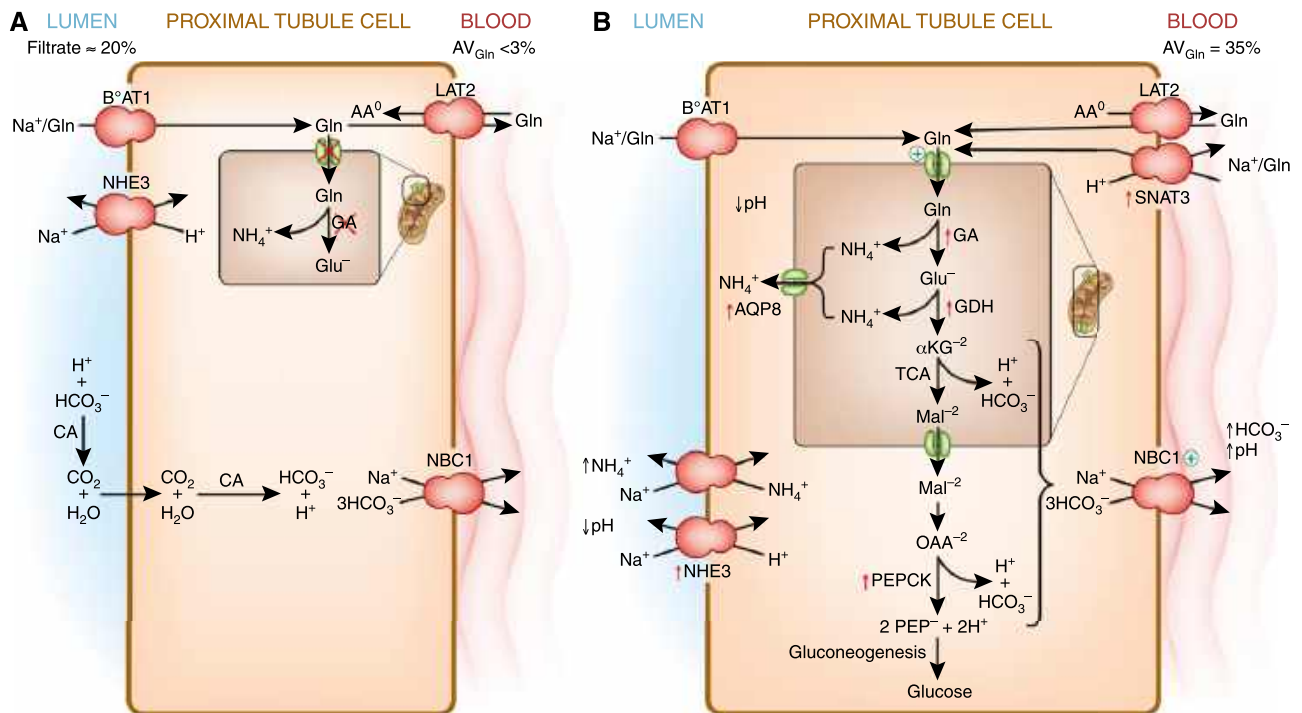


Figure 5. | Renal proximal tubular catabolism of glutamine. (A) During normal acid-base balance, the glutamine filtered by the glomeruli is nearly quantitatively extracted from the lumen of the proximal convoluted tubule and largely returned to the blood. The transepithelial transport utilizes B⁰AT1, a Na⁺-dependent neutral amino acid cotransporter in the apical membrane, and LAT2, a neutral amino acid antiporter in the basolateral membrane. To accomplish this movement, either the mitochondrial glutamine transporter or the mitochondrial glutaminase (GA) must be inhibited (red X). The apical Na⁺/H⁺ exchanger functions to slightly acidify the lumen to facilitate the recovery of HCO₃⁻ ions. (B) During chronic acidosis, approximately one third of the plasma glutamine is extracted and catabolized within the early portion of the proximal tubule. B⁰AT1 continues to mediate the extraction of glutamine from the lumen. Uptake of glutamine through the basolateral membrane occurs by reversal of the neutral amino acid exchanger, LAT2, and through increased expression of SNAT3. Increased renal catabolism of glutamine is facilitated by increased expression (red arrows) of the genes that encode glutaminase (GA), glutamate dehydrogenase (GDH), phosphoenolpyruvate carboxykinase (PEPCK), the mitochondrial aquaporin-8 (AQP8), the apical Na⁺/H⁺ exchanger (NHE3), and the basolateral glutamine transporter (SNAT3). In addition, the activities of the mitochondrial glutamine transporter and the basolateral Na⁺/3HCO₃⁻ are increased (+). Increased expression of NHE3 contributes to the transport of ammonium ions and the acidification of the luminal fluid. The combined increases in renal ammonium ion excretion and gluconeogenesis result in a net synthesis of HCO₃⁻ ions that are transported across the basolateral membrane by the Na⁺/3HCO₃⁻ cotransporter (NBC1). CA, carbonic anhydrase; αKG, α-ketoglutarate; Mal, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate.

synthetase (75). Thus, the SNAT3 transporter may normally facilitate the pH-dependent release of glutamine. However, during chronic acidosis, increased expression of the SNAT3 transporter occurs primarily in the basolateral membranes of the S1 and S2 segments of proximal tubule, the site of increased glutamine catabolism (76). Given the sustained increase in H⁺ ion concentration within these cells, the increased expression of the SNAT3 transporter may now facilitate the basolateral uptake of glutamine and contribute to its sustained extraction during chronic acidosis. Within 8–24 hours after onset of acidosis, a pronounced increase in phosphoenolpyruvate carboxykinase (77) also occurs only in the proximal convoluted tubule. More gradual increases in levels of mitochondrial glutaminase (78,79) and glutamate dehydrogenase (80) that require 4–7 days also occur solely within the proximal convoluted tubule. In addition, the level of aquaporin-8, a potential mitochondrial ammonia transporter, is increased (81).

Glutamine uptake in mitochondria from normal rats is mediated through two glutamine antiporters, whereas a

highly active glutamine uniporter is evident only in mitochondria prepared from acidotic rats (82). Therefore, acidosis leads to increased expression or activation of a unique, but unidentified, mitochondrial glutamine transporter. Acute activation and subsequent increase in expression of NHE3 (83–85) acidifies the fluid in the tubular lumen and contributes to the active transport of ammonium ions as a direct substrate of NHE3 (69). The adaptation in NHE3 likely reflects the increased demand for ammonium ion secretion although this is difficult to prove. The filtered HCO₃⁻ load is certainly decreased in metabolic acidosis, so increased HCO₃⁻ absorptive capacity is not required. As a result, increased renal ammoniogenesis continues to provide an expendable cation that facilitates excretion of strong acids while conserving sodium and potassium ions. In rats (86,87) and humans (35), the α-ketoglutarate generated from renal catabolism of glutamine is primarily converted to glucose. This process requires phosphoenolpyruvate carboxykinase to divert oxaloacetate, derived from intermediates of the tricarboxylic acid cycle, into the pathway of gluconeogenesis. The combined

pathways of ammoniagenesis and gluconeogenesis result in a net production of 2 NH_4^+ and 2 HCO_3^- ions per glutamine. Activation of NBCe1A (83), the basolateral $\text{Na}^+/\text{3HCO}_3^-$ cotransporter, facilitates the translocation of reabsorbed and *de novo*-synthesized HCO_3^- ions into the renal venous blood. Thus, the combined adaptations also create a net renal release of HCO_3^- ions that partially restores acid-base balance.

The adaptative increase in the NaDC-1 transporter contributes to increased reabsorption and metabolism of citrate within the proximal tubule. This reduces the excretion of a valuable base in the urine. This adaptation occurs at multiple levels. Acidification of luminal pH titrates citrate³⁻ to citrate²⁻, which is the preferred substrate, and low pH also directly activates NaDC1 to increase transport independent of [citrate²⁻] (Figure 3B) (26,88). In addition to transport, increased cellular metabolism also drives citrate reabsorption. After cellular uptake, citrate is metabolized through one of two pathways: a cytoplasmic pathway involving citrate lyase or a mitochondrial pathway involving the citric acid cycle (47). During acidosis, the activities of cytoplasmic citrate lyase and mitochondrial aconitase are also increased (89). Because both pathways generate HCO_3^- , the increased reabsorption of citrate is equivalent to a decrease in base excretion (90). Enhanced catabolism of citrate also produces substrates that support the increased gluconeogenesis.

Acid-base disturbances are a major regulator of proximal tubule phosphate handling. Metabolic and respiratory acidosis increase phosphate excretion and metabolic and respiratory alkalosis decrease phosphate excretion. The mechanism of phosphaturia in acidosis is complex and mediated by many factors, including increased release of phosphate from bone (91), titration of luminal phosphate to monovalent form, direct gating of NaPi-IIa and NaPi-IIc by pH (54), decrease in apical membrane protein (92), and transcripts (93) of the transporters, although disparate results on protein levels have been described (Figure 3B) (94). The acid-induced phosphaturia serves to increase urinary H^+ buffer but also accommodates the phosphate released from bone associated with acid loading.

The gradual increases in glutaminase (95–97) and glutamate dehydrogenase (98) result from selective stabilization of their mRNAs. By contrast, the rapid increase in phosphoenolpyruvate carboxykinase results from enhanced transcription of the *PCK-1* gene (99), whereas mRNA stabilization contributes to the sustained increase (100,101). The presence of a pH-response element (pH-RE) that regulates the turnover of the glutaminase mRNA was initially demonstrated by stable expression of a chimeric β -globin reporter mRNA, which includes a 956-bp segment of the 3'-untranslated region of glutaminase mRNA (95). RNA gel shift analyses of various deletion constructs mapped the pH-RE to two 8-nt AU-sequences within the 3'-untranslated region. Mutation of the pH-RE within the β -globin reporter mRNA blocked the pH-responsive stabilization. In addition, insertion of only a 29-bp segment containing the pH-RE was sufficient to produce both rapid degradation and pH-responsive stabilization (102). Therefore, the identified pH-RE contributes to the rapid turnover of the glutaminase mRNA and is both necessary and sufficient to mediate its pH-responsive stabilization.

Proteomic analysis of isolated rat renal proximal convoluted tubules identified an additional 60 proteins that are increased during acute and chronic acidosis (103–105). More than 50% of the mRNAs that encode the induced proteins contain an AU-sequence that has >85% identity with the pH-response element found in the glutaminase mRNA. This finding strongly suggests that mRNA stabilization is a primary mechanism by which protein expression is increased in response to onset of metabolic acidosis.

The maintenance of the composition and content of the extracellular fluid volume is critical to health, as evidenced by the multiple organ failure seen in kidney disease. The mammalian proximal tubule uses an array of polarized membrane transporters to accomplish vectorial solute transport. By lowering the luminal content of many abundant solutes to a level that is within the lower reabsorptive capacity of the distal nephron segments, it enables the absorption of these solutes to be fine-tuned downstream. Without the large proximal reabsorption capacity, it would be impossible for the kidney to maintain the high GFR that is necessary to sustain the high metabolic rates characteristic of terrestrial vertebrates. For many solutes with lower plasma concentrations and, hence, lower filtered load, proximal reabsorption is the final arbitrator of urinary excretion. The kidney has enormous capacity to cope with a wide range of physiologic challenges. Whereas volatile acids can be excreted in a gaseous phase in the lung, the kidney is the only organ where nonvolatile acids can be excreted and where decomposed body buffers, such as bicarbonate, can be regenerated. Metabolic acid loading and metabolic acidosis have undesirable acute and chronic consequences and they trigger a coordinated multiorgan network of adaptive responses that partially rectify the disturbance. Although intuitive in principle, the actual mechanisms are actually extremely complex and the proximal tubule is in the center stage spotlight. With a built-in cast of players inherent to this epithelium, the proximal tubule elicits a complex set of mechanisms that includes intrinsic pH sensing and adaptations in membrane transporters and metabolic enzymes to take a neutral molecule, partition it into an acid and a base, and transfer the acid into urine and the base into the blood. Although the short account in this article summarizes significant advances made over decades, the understanding of this system is just beginning. With the current upsurge of powerful investigative methods, the delineation of mechanisms of adaptation to acidosis will emerge with greater rapidity and clarity.

Acknowledgments

This study was supported in part by grants from the National Institutes of Health (DK037124 to N.P.C. and AI041612, DK078596, and DK091392 to O.W.M.). O.W.M. also received support from the O'Brien Kidney Research Center (DK-079328) and Genzyme.

Disclosures

N.P.C. is a consultant for Calithera and O.W.M. has received research support from Genzyme.

References

1. Jones G, Prosser DE, Kaufmann M: 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): Its important role in the degradation of vitamin D. *Arch Biochem Biophys* 523: 9–18, 2012

2. Meyer C, Dostou JM, Gerich JE: Role of the human kidney in glucose counterregulation. *Diabetes* 48: 943–948, 1999
3. De Vito E, Cabrera RR, Fasciolo JC: Renin production and release by rat kidney slices. *Am J Physiol* 219: 1042–1045, 1970
4. Koury ST, Bondurant MC, Koury MJ: Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. *Blood* 71: 524–527, 1988
5. Moe OW, Ujiiie K, Star RA, Miller RT, Widell J, Alpern RJ, Henrich WL: Renin expression in renal proximal tubule. *J Clin Invest* 91: 774–779, 1993
6. Moe OW, Giebisch G, Seldin DW: The logic of the kidney. In: *Genetic Diseases of the Kidney*, edited by Lifton R, Somlo S, Seldin DW, Giebisch G, Amsterdam, Elsevier, 2009, pp 29–76
7. Aronson PS: Ion exchangers mediating NaCl transport in the renal proximal tubule. *Cell Biochem Biophys* 36: 147–153, 2002
8. Moe OW, Preisig PA, Alpern RJ: Cellular model of proximal tubule NaCl and NaHCO₃ absorption. *Kidney Int* 38: 605–611, 1990
9. Preisig PA, Rector FC Jr: Role of Na⁺-H⁺ antiport in rat proximal tubule NaCl absorption. *Am J Physiol* 255: F461–F465, 1988
10. Aronson PS, Sacktor B: The Na⁺ gradient-dependent transport of D-glucose in renal brush border membranes. *J Biol Chem* 250: 6032–6039, 1975
11. Rector FC Jr: Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am J Physiol* 244: F461–F471, 1983
12. Harris PJ, Young JA: Dose-dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pflügers Arch* 367: 295–297, 1977
13. Garcia NH, Garvin JL: Endothelin's biphasic effect on fluid absorption in the proximal straight tubule and its inhibitory cascade. *J Clin Invest* 93: 2572–2577, 1994
14. Gill JR Jr, Casper AG: Effect of renal alpha-adrenergic stimulation on proximal tubular sodium reabsorption. *Am J Physiol* 223: 1201–1205, 1972
15. Baum M, Quigley R: Inhibition of proximal convoluted tubule transport by dopamine. *Kidney Int* 54: 1593–1600, 1998
16. Moe OW, Tejedor A, Levi M, Seldin DW, Preisig PA, Alpern RJ: Dietary NaCl modulates Na⁺-H⁺ antiporter activity in renal cortical apical membrane vesicles. *Am J Physiol* 260: F130–F137, 1991
17. Bobulescu IA, Moe OW: Luminal Na⁺/H⁺ exchange in the proximal tubule. *Pflügers Arch* 458: 5–21, 2009
18. Bobulescu IA, Moe OW: Na⁺/H⁺ exchangers in renal regulation of acid-base balance. *Semin Nephrol* 26: 334–344, 2006
19. Hamm LL: Renal handling of citrate. *Kidney Int* 38: 728–735, 1990
20. DuBose TD Jr, Pucacco LR, Seldin DW, Carter NW, Kokko JP: Microelectrode determination of pH and PCO₂ in rat proximal tubule after benzolamide: Evidence for hydrogen ion secretion. *Kidney Int* 15: 624–629, 1979
21. Chantrelle B, Cogan MG, Rector FC Jr: Evidence for coupled sodium/hydrogen exchange in the rat superficial proximal convoluted tubule. *Pflügers Arch* 395: 186–189, 1982
22. Preisig PA, Ives HE, Cragoe EJ Jr, Alpern RJ, Rector FC Jr: Role of the Na⁺/H⁺ antiporter in rat proximal tubule bicarbonate absorption. *J Clin Invest* 80: 970–978, 1987
23. Amemiya M, Loffing J, Lötscher M, Kaissling B, Alpern RJ, Moe OW: Expression of NHE-3 in the apical membrane of rat renal proximal tubule and thick ascending limb. *Kidney Int* 48: 1206–1215, 1995
24. Becker AM, Zhang J, Goyal S, Dwarakanath V, Aronson PS, Moe OW, Baum M: Ontogeny of NHE8 in the rat proximal tubule. *Am J Physiol Renal Physiol* 293: F255–F261, 2007
25. Nagami GT: Luminal secretion of ammonia in the mouse proximal tubule perfused in vitro. *J Clin Invest* 81: 159–164, 1988
26. Brennan S, Hering-Smith K, Hamm LL: Effect of pH on citrate reabsorption in the proximal convoluted tubule. *Am J Physiol* 255: F301–F306, 1988
27. Hamm LL, Simon EE: Roles and mechanisms of urinary buffer excretion. *Am J Physiol* 253: F595–F605, 1987
28. Boron WF, Boulpaep EL: Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO₃⁻ transport. *J Gen Physiol* 81: 53–94, 1983
29. Schmitt BM, Biemesderfer D, Romero MF, Boulpaep EL, Boron WF: Immunolocalization of the electrogenic Na⁺-HCO₃⁻ co-transporter in mammalian and amphibian kidney. *Am J Physiol* 276: F27–F38, 1999
30. Hummel CS, Lu C, Loo DD, Hirayama BA, Voss AA, Wright EM: Glucose transport by human renal Na⁺/D-glucose co-transporters SGLT1 and SGLT2. *Am J Physiol Cell Physiol* 300: C14–C21, 2011
31. Vallon V, Platt KA, Cunard R, Schroth J, Whaley J, Thomson SC, Koepsell H, Rieg T: SGLT2 mediates glucose reabsorption in the early proximal tubule. *J Am Soc Nephrol* 22: 104–112, 2011
32. Bakris GL, Fonseca VA, Sharma K, Wright EM: Renal sodium-glucose transport: Role in diabetes mellitus and potential clinical implications. *Kidney Int* 75: 1272–1277, 2009
33. Vallon V, Rose M, Gerasimova M, Satriano J, Platt KA, Koepsell H, Cunard R, Sharma K, Thomson SC, Rieg T: Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. *Am J Physiol Renal Physiol* 304: F156–F167, 2013
34. Vandewalle A, Wirthensohn G, Heidrich HG, Guder WG: Distribution of hexokinase and phosphoenolpyruvate carboxykinase along the rabbit nephron. *Am J Physiol* 240: F492–F500, 1981
35. Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: Its importance in human glucose homeostasis. *Diabetes Care* 24: 382–391, 2001
36. McGivan JD, Bungard CI: The transport of glutamine into mammalian cells. *Front Biosci* 12: 874–882, 2007
37. Verrey F, Singer D, Ramadan T, Vuille-dit-Bille RN, Mariotta L, Camargo SM: Kidney amino acid transport. *Pflügers Arch* 458: 53–60, 2009
38. Romeo E, Dave MH, Bacic D, Ristic Z, Camargo SM, Loffing J, Wagner CA, Verrey F: Luminal kidney and intestine SLC6 amino acid transporters of B0AT-cluster and their tissue distribution in *Mus musculus*. *Am J Physiol Renal Physiol* 290: F376–F383, 2006
39. Silbernagl S: Tubular reabsorption of L-glutamine studied by free-flow micropuncture and microperfusion of rat kidney. *Int J Biochem* 12: 9–16, 1980
40. Kleta R, Romeo E, Ristic Z, Ohura T, Stuart C, Arcos-Burgos M, Dave MH, Wagner CA, Camargo SR, Inoue S, Matsuura N, Helip-Wooley A, Bockenhauer D, Warth R, Bernardini I, Visser G, Eggemann T, Lee P, Chairoungdua A, Jutabha P, Babu E, Nilwarangkoon S, Anzai N, Kanai Y, Verrey F, Gahl WA, Koizumi A: Mutations in SLC6A19, encoding B0AT1, cause Hartnup disorder. *Nat Genet* 36: 999–1002, 2004
41. Seow HF, Bröer S, Bröer A, Bailey CG, Potter SJ, Cavanaugh JA, Rasko JE: Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. *Nat Genet* 36: 1003–1007, 2004
42. Shayakul C, Kanai Y, Lee WS, Brown D, Rothstein JD, Hediger MA: Localization of the high-affinity glutamate transporter EAAC1 in rat kidney. *Am J Physiol* 273: F1023–F1029, 1997
43. Rossier G, Meier C, Bauch C, Summa V, Sordat B, Verrey F, Kühn LC: LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. *J Biol Chem* 274: 34948–34954, 1999
44. Torrents D, Estévez R, Pineda M, Fernández E, Lloberas J, Shi YB, Zorzano A, Palacin M: Identification and characterization of a membrane protein (y⁺L amino acid transporter-1) that associates with 4F2hc to encode the amino acid transport activity y⁺L. A candidate gene for lysinuric protein intolerance. *J Biol Chem* 273: 32437–32445, 1998
45. Moe OW, Palacin M, Wright S: *Renal Organic Solute Transport*, Philadelphia, Saunders Elsevier, 2007
46. Pajor AM: Sequence and functional characterization of a renal sodium/dicarboxylate cotransporter. *J Biol Chem* 270: 5779–5785, 1995
47. Simpson DP: Citrate excretion: A window on renal metabolism. *Am J Physiol* 244: F223–F234, 1983
48. Moe OW, Preisig PA: Dual role of citrate in mammalian urine. *Curr Opin Nephrol Hypertens* 15: 419–424, 2006
49. Moe OW: Kidney stones: Pathophysiology and medical management. *Lancet* 367: 333–344, 2006

50. Hu MC, Shiizaki K, Kuro-o M, Moe OW: Fibroblast growth factor 23 and Klotho: Physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* 75: 503–533, 2013
51. Marks J, Debnam ES, Unwin RJ: Phosphate homeostasis and the renal-gastrointestinal axis. *Am J Physiol Renal Physiol* 299: F285–F296, 2010
52. Biber J, Hernando N, Forster I, Murer H: Regulation of phosphate transport in proximal tubules. *Pflügers Arch* 458: 39–52, 2009
53. Peraino RA, Suki WN: Phosphate transport by isolated rabbit cortical collecting tubule. *Am J Physiol* 238: F358–F362, 1980
54. Virkik LV, Biber J, Murer H, Forster IC: Phosphate transporters: A tale of two solute carrier families. *Am J Physiol Renal Physiol* 293: F643–F654, 2007
55. Haramati A, Haas JA, Knox FG: Nephron heterogeneity of phosphate reabsorption: Effect of parathyroid hormone. *Am J Physiol* 246: F155–F158, 1984
56. Glahn RP, Onsgard MJ, Tyce GM, Chinnow SL, Knox FG, Dousa TP: Autocrine/paracrine regulation of renal Na(+)-phosphate cotransport by dopamine. *Am J Physiol* 264: F618–F622, 1993
57. Gattineni J, Bates C, Twombly K, Dwarakanath V, Robinson ML, Goetz R, Mohammadi M, Baum M: FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF receptor 1. *Am J Physiol Renal Physiol* 297: F282–F291, 2009
58. Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, Razzaque MS, Rosenblatt KP, Baum MG, Kuro-o M, Moe OW: Klotho: A novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 24: 3438–3450, 2010
59. Berndt T, Thomas LF, Craig TA, Sommer S, Li X, Bergstralh EJ, Kumar R: Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. *Proc Natl Acad Sci U S A* 104: 11085–11090, 2007
60. Pfister MF, Hilfiker H, Forgo J, Lederer E, Biber J, Murer H: Cellular mechanisms involved in the acute adaptation of OK cell Na/Pi-cotransport to high- or low-Pi medium. *Pflügers Arch* 435: 713–719, 1998
61. Squires EJ, Hall DE, Brosnan JT: Arteriovenous differences for amino acids and lactate across kidneys of normal and acidotic rats. *Biochem J* 160: 125–128, 1976
62. Meyer C, Stumvoll M, Dostou J, Welle S, Haymond M, Gerich J: Renal substrate exchange and gluconeogenesis in normal postabsorptive humans. *Am J Physiol Endocrinol Metab* 282: E428–E434, 2002
63. Tamarappoo BK, Joshi S, Welbourne TC: Interorgan glutamine flow regulation in metabolic acidosis. *Miner Electrolyte Metab* 16: 322–330, 1990
64. Hughey RP, Rankin BB, Curthoys NP: Acute acidosis and renal arteriovenous differences of glutamine in normal and adrenalectomized rats. *Am J Physiol* 238: F199–F204, 1980
65. Schröck H, Cha CJ, Goldstein L: Glutamine release from hindlimb and uptake by kidney in the acutely acidotic rat. *Biochem J* 188: 557–560, 1980
66. Sastrasin M, Sastrasin S: Effect of acute pH change on mitochondrial glutamine transport. *Am J Physiol* 259: F863–F866, 1990
67. Yang X, Amemiya M, Peng Y, Moe OW, Preisig PA, Alpern RJ: Acid incubation causes exocytic insertion of NHE3 in OKP cells. *Am J Physiol Cell Physiol* 279: C410–C419, 2000
68. Horie S, Moe O, Tejedor A, Alpern RJ: Preincubation in acid medium increases Na/H antiporter activity in cultured renal proximal tubule cells. *Proc Natl Acad Sci U S A* 87: 4742–4745, 1990
69. Tannen RL, Ross BD: Ammoniogenesis by the isolated perfused rat kidney: The critical role of urinary acidification. *Clin Sci (Lond)* 56: 353–364, 1979
70. Lowry M, Ross BD: Activation of oxoglutarate dehydrogenase in the kidney in response to acute acidosis. *Biochem J* 190: 771–780, 1980
71. Moret C, Dave MH, Schulz N, Jiang JX, Verrey F, Wagner CA: Regulation of renal amino acid transporters during metabolic acidosis. *Am J Physiol Renal Physiol* 292: F555–F566, 2007
72. Christensen HN: Role of amino acid transport and counter-transport in nutrition and metabolism. *Physiol Rev* 70: 43–77, 1990
73. Chaudhry FA, Reimer RJ, Edwards RH: The glutamine commute: Take the N line and transfer to the A. *J Cell Biol* 157: 349–355, 2002
74. Karinch AM, Lin CM, Wolfgang CL, Pan M, Souba WW: Regulation of expression of the SN1 transporter during renal adaptation to chronic metabolic acidosis in rats. *Am J Physiol Renal Physiol* 283: F1011–F1019, 2002
75. Taylor L, Curthoys NP: Glutamine metabolism: Role in acid-base balance. *Biochem Mol Biol Educ* 32: 291–304, 2004
76. Solbu TT, Boulland JL, Zahid W, Lyamouri Bredahl MK, Amiry-Moghaddam M, Storm-Mathisen J, Roberg BA, Chaudhry FA: Induction and targeting of the glutamine transporter SN1 to the basolateral membranes of cortical kidney tubule cells during chronic metabolic acidosis suggest a role in pH regulation. *J Am Soc Nephrol* 16: 869–877, 2005
77. Schoolwerth AC, deBoer PA, Moorman AF, Lamers WH: Changes in mRNAs for enzymes of glutamine metabolism in kidney and liver during ammonium chloride acidosis. *Am J Physiol* 267: F400–F406, 1994
78. Curthoys NP, Lowry OH: The distribution of glutaminase isoenzymes in the various structures of the nephron in normal, acidotic, and alkalotic rat kidney. *J Biol Chem* 248: 162–168, 1973
79. Wright PA, Knepper MA: Phosphate-dependent glutaminase activity in rat renal cortical and medullary tubule segments. *Am J Physiol* 259: F961–F970, 1990
80. Wright PA, Knepper MA: Glutamate dehydrogenase activities in microdissected rat nephron segments: Effects of acid-base loading. *Am J Physiol* 259: F53–F59, 1990
81. Molinas SM, Trumper L, Marinelli RA: Mitochondrial aquaporin-8 in renal proximal tubule cells: Evidence for a role in the response to metabolic acidosis. *Am J Physiol Renal Physiol* 303: F458–F466, 2012
82. Atlante A, Passarella S, Minervini GM, Quagliariello E: Glutamine transport in normal and acidotic rat kidney mitochondria. *Arch Biochem Biophys* 315: 369–381, 1994
83. Preisig PA, Alpern RJ: Chronic metabolic acidosis causes an adaptation in the apical membrane Na/H antiporter and basolateral membrane Na(HCO₃)₃ symporter in the rat proximal convoluted tubule. *J Clin Invest* 82: 1445–1453, 1988
84. Ambühl PM, Amemiya M, Danczkay M, Lötscher M, Kaissling B, Moe OW, Preisig PA, Alpern RJ: Chronic metabolic acidosis increases NHE3 protein abundance in rat kidney. *Am J Physiol* 271: F917–F925, 1996
85. Wu MS, Biemesderfer D, Giebisch G, Aronson PS: Role of NHE3 in mediating renal brush border Na⁺-H⁺ exchange. Adaptation to metabolic acidosis. *J Biol Chem* 271: 32749–32752, 1996
86. Pagliara AS, Goodman AD: Relation of renal cortical gluconeogenesis, glutamate content, and production of ammonia. *J Clin Invest* 49: 1967–1974, 1970
87. Roobol A, Alleyne GA: Control of renal cortex ammoniogenesis and its relationship to renal cortex gluconeogenesis. *Biochim Biophys Acta* 362: 83–91, 1974
88. Wright SH, Kippen I, Wright EM: Effect of pH on the transport of Krebs cycle intermediates in renal brush border membranes. *Biochim Biophys Acta* 684: 287–290, 1982
89. Aruga S, Wehrli S, Kaissling B, Moe OW, Preisig PA, Pajor AM, Alpern RJ: Chronic metabolic acidosis increases NaDC-1 mRNA and protein abundance in rat kidney. *Kidney Int* 58: 206–215, 2000
90. Preisig PA: The acid-activated signaling pathway: Starting with Pyk2 and ending with increased NHE3 activity. *Kidney Int* 72: 1324–1329, 2007
91. Lemann J Jr, Bushinsky DA, Hamm LL: Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol* 285: F811–F832, 2003
92. Ambühl PM, Zajicek HK, Wang H, Puttaparthi K, Levi M: Regulation of renal phosphate transport by acute and chronic

- metabolic acidosis in the rat. *Kidney Int* 53: 1288–1298, 1998
93. Jehle AW, Hilfiker H, Pfister MF, Biber J, Lederer E, Krapf R, Murer H: Type II Na-Pi cotransport is regulated transcriptionally by ambient bicarbonate/carbon dioxide tension in OK cells. *Am J Physiol* 276: F46–F53, 1999
 94. Nowik M, Picard N, Stange G, Capuano P, Tenenhouse HS, Biber J, Murer H, Wagner CA: Renal phosphaturia during metabolic acidosis revisited: Molecular mechanisms for decreased renal phosphate reabsorption. *Pflügers Arch* 457: 539–549, 2008
 95. Hansen WR, Barsic-Tress N, Taylor L, Curthoys NP: The 3'-nontranslated region of rat renal glutaminase mRNA contains a pH-responsive stability element. *Am J Physiol* 271: F126–F131, 1996
 96. Laterza OF, Hansen WR, Taylor L, Curthoys NP: Identification of an mRNA-binding protein and the specific elements that may mediate the pH-responsive induction of renal glutaminase mRNA. *J Biol Chem* 272: 22481–22488, 1997
 97. Laterza OF, Curthoys NP: Specificity and functional analysis of the pH-responsive element within renal glutaminase mRNA. *Am J Physiol Renal Physiol* 278: F970–F977, 2000
 98. Schroeder JM, Liu W, Curthoys NP: pH-responsive stabilization of glutamate dehydrogenase mRNA in LLC-PK1-F+ cells. *Am J Physiol Renal Physiol* 285: F258–F265, 2003
 99. Hanson RW, Reshef L: Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem* 66: 581–611, 1997
 100. Hwang JJ, Perera S, Shapiro RA, Curthoys NP: Mechanism of altered renal glutaminase gene expression in response to chronic acidosis. *Biochemistry* 30: 7522–7526, 1991
 101. Mufti J, Hajarnis S, Shepardson K, Gummadi L, Taylor L, Curthoys NP: Role of AUF1 and HuR in the pH-responsive stabilization of phosphoenolpyruvate carboxykinase mRNA in LLC-PK1-F+ cells. *Am J Physiol Renal Physiol* 301: F1066–F1077, 2011
 102. Schroeder JM, Ibrahim H, Taylor L, Curthoys NP: Role of deadenylation and AUF1 binding in the pH-responsive stabilization of glutaminase mRNA. *Am J Physiol Renal Physiol* 290: F733–F740, 2006
 103. Curthoys NP, Taylor L, Hoffert JD, Knepper MA: Proteomic analysis of the adaptive response of rat renal proximal tubules to metabolic acidosis. *Am J Physiol Renal Physiol* 292: F140–F147, 2007
 104. Freund DM, Prenni JE, Curthoys NP: Response of the mitochondrial proteome of rat renal proximal convoluted tubules to chronic metabolic acidosis. *Am J Physiol Renal Physiol* 304: F145–F155, 2013
 105. Walmsley SJ, Freund DM, Curthoys NP: Proteomic profiling of the effect of metabolic acidosis on the apical membrane of the proximal convoluted tubule. *Am J Physiol Renal Physiol* 302: F1465–F1477, 2012

Published online ahead of print. Publication date available at www.cjasn.org.



Urine-Concentrating Mechanism in the Inner Medulla: Function of the Thin Limbs of the Loops of Henle

William H. Dantzler,* Anita T. Layton,[†] Harold E. Layton,[†] and Thomas L. Pannabecker*

Summary

The ability of mammals to produce urine hyperosmotic to plasma requires the generation of a gradient of increasing osmolality along the medulla from the corticomedullary junction to the papilla tip. Countercurrent multiplication apparently establishes this gradient in the outer medulla, where there is substantial transepithelial reabsorption of NaCl from the water-impermeable thick ascending limbs of the loops of Henle. However, this process does not establish the much steeper osmotic gradient in the inner medulla, where there are no thick ascending limbs of the loops of Henle and the water-impermeable ascending thin limbs lack active transepithelial transport of NaCl or any other solute. The mechanism generating the osmotic gradient in the inner medulla remains an unsolved mystery, although it is generally considered to involve countercurrent flows in the tubules and vessels. A possible role for the three-dimensional interactions between these inner medullary tubules and vessels in the concentrating process is suggested by creation of physiologic models that depict the three-dimensional relationships of tubules and vessels and their solute and water permeabilities in rat kidneys and by creation of mathematical models based on biologic phenomena. The current mathematical model, which incorporates experimentally determined or estimated solute and water flows through clearly defined tubular and interstitial compartments, predicts a urine osmolality in good agreement with that observed in moderately antidiuretic rats. The current model provides substantially better predictions than previous models; however, the current model still fails to predict urine osmolalities of maximally concentrating rats.

Clin J Am Soc Nephrol 9: 1781–1789, 2014. doi: 10.2215/CJN.08750812

Introduction

Humans, like most mammals, are capable of producing urine that is hyperosmotic to their plasma, thereby enabling them to excrete solutes with a minimal loss of water, a process clearly essential to human health and normal daily activity. This urine-concentrating process involves generation of a gradient of increasing osmolality along the renal medulla from the corticomedullary junction to the tip of the papilla (as illustrated for the rat kidney in Figure 1) (1–3). This osmotic gradient is formed by the accumulation of solutes, primarily NaCl and urea, in the cells, interstitium, tubules, and vessels of the medulla (4–6). In the presence of antidiuretic hormone (arginine vasopressin), the osmotic water permeability of the collecting duct (CD) epithelium increases, water moves from the CDs into the more concentrated interstitium, and the osmolality of the CD fluid increases, nearly equaling the osmolality of the surrounding interstitium in maximum antidiuresis.

The mechanism by which the medullary osmotic gradient is generated is only partially understood. Between 1940 and 1960, a series of experimental and theoretical studies (6–9) appeared to provide a mechanism for generating this gradient. These studies indicated that a small osmotic pressure difference between the ascending and descending limbs of the loops of Henle, generated by net transport of solute, unaccompanied by water, out of the ascending limbs could be multiplied by countercurrent flow in the

loops to generate the corticopapillary osmotic gradient (Figure 1). Washout of this gradient would be mitigated by countercurrent exchange in the vasa recta. The processes of countercurrent multiplication and countercurrent exchange for generating and maintaining the medullary osmotic gradient, respectively, are emphasized in most texts on renal physiology.

The classic concept of countercurrent multiplication, although recently challenged (10), has been widely accepted as generating the osmotic gradient in the outer medulla. Here, the well documented active transport of NaCl out of the water-impermeable thick ascending limbs (TALs) (11,12) has been shown to be theoretically sufficient to generate the observed gradient (13) (Figure 1).

The classic countercurrent multiplication hypothesis, however, has not been accepted for the inner medulla (IM), where the steepest part of the osmotic gradient is generated (Figure 1) but where there are no TALs of the loops of Henle. Although the ascending thin limbs (ATLs) in the IM, like the TALs in the outer medulla, have essentially no transepithelial osmotic water permeability (14–17), they also have no known active transepithelial transport of NaCl or any other solute (16,18–21). If this steep osmotic gradient in the IM is not generated by the same mechanism as the gradient in the outer medulla, how, then, is it generated? Most scientists who study the urine-concentrating process believe that some form of countercurrent multiplication

*Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona; and

[†]Department of Mathematics, Duke University, Durham, North Carolina

Correspondence:

Dr. William H. Dantzler, University of Arizona Health Sciences Center, Department of Physiology, AHSC 4130A, 1501 N. Campbell Avenue, Tucson, AZ 85724-5051. Email: dantzler@Email.arizona.edu

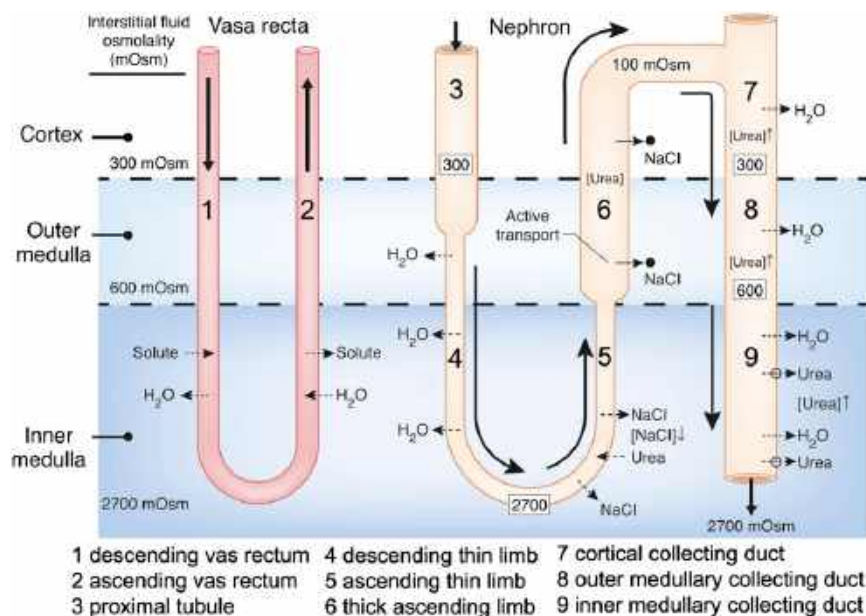


Figure 1. | Diagram of a single vasa rectum and single long-looped nephron, illustrating how classic countercurrent multiplication could produce the osmotic gradient in the outer medulla, and how the “passive mechanism” was proposed to produce the osmotic gradient in the inner medulla (23,24). In the outer medulla, active transport of NaCl out of the water-impermeable thick ascending limb (indicated by solid arrows and black dots) creates the small osmotic pressure difference between that limb and the descending limb sufficient for classic countercurrent multiplication to generate the osmotic gradient (approximate osmolalities for rat kidney given by numbers in the black boxes). For the proposed “passive” mechanism in the inner medulla, urea ($[urea]^t$) is concentrated ($[urea]^t$) in urea-impermeable cortical and outer medullary collecting ducts by reabsorption of NaCl and water (broken arrows indicate passive movement). Urea then moves passively out of the inner medullary collecting ducts via vasopressin-regulated urea transporters (UT-A1, UT-A3; open circles and broken lines) into the surrounding inner medulla interstitium. This increased concentration of urea in the inner medullary interstitium draws water from descending thin limbs (DTLs) and inner medullary collecting ducts (IMCDs), thereby reducing inner medullary interstitial concentration of NaCl ($[NaCl]^i$). These processes result in a urea concentration in the interstitium that is higher than the urea concentration in the ascending thin limbs (ATLs) and a NaCl concentration in the ATLs that is higher than the NaCl concentration in the interstitium. NaCl will then tend to diffuse out of the ATLs (broken arrows) and urea will tend to diffuse into the ATLs (broken arrows). If the permeability of the ATLs to NaCl is sufficiently high and to urea sufficiently low, the interstitial fluid will be concentrated (producing the osmotic gradient) as the ATL fluid is being diluted. Countercurrent exchange of solutes and water helping to preserve this gradient is indicated in the vasa rectum. Segments are numbered according to key.

involving the thin limbs generates the gradient. However, there is no accepted mechanism for how a countercurrent multiplication system might work.

The most influential and frequently cited explanation of the urine-concentrating mechanism in the IM is the “passive mechanism” hypothesis proposed simultaneously and independently in 1972 by Kokko and Rector (22) and Stephenson (23). They hypothesized that separation of NaCl from urea by active transport of NaCl (without urea or water) out of the TAL in the outer medulla provides a source of potential energy for generating an osmotic gradient in the IM (Figure 1). They suggested that urea, which has been concentrated in the CDs in the cortex and outer medulla by reabsorption of NaCl and water, diffuses out of the inner medullary CDs (IMCDs) into the surrounding inner medullary interstitium. This increased inner medullary interstitial urea concentration, in turn, draws water from the descending thin limbs (DTLs) and IMCDs, thereby reducing the inner medullary interstitial concentration of NaCl. These processes result in a urea concentration in the interstitium that is higher than the urea concentration in the ATLs, and a NaCl concentration in the ATLs that is higher than the NaCl concentration in the interstitium. NaCl will then tend to

diffuse out of the ATLs into the interstitium, and urea will tend to diffuse from the interstitium into the ATLs. If the permeability of the ATLs to NaCl is sufficiently high and to urea sufficiently low, the interstitial fluid will be concentrated as the ATL fluid is being diluted. The increased interstitial osmolality will, in turn, cause water to move out of the CDs, thereby concentrating the urine. We consider this a “solute-separation, solute-mixing” mechanism: “solute-separation” referring to active NaCl reabsorption without urea in TALs in the outer medulla, and “solute-mixing” referring to mixing of urea diffusing from CDs with NaCl diffusing from the ATLs in the IM (24) (Figure 1).

This appears to be an elegant model and is described in most renal texts, but its function, as noted above, depends critically on the transepithelial permeabilities of the ATLs to NaCl and urea. Unfortunately, using available measurements of the urea permeabilities, no one has been able to develop a mathematical model that generates a significant axial osmotic gradient in the IM with this initial version of the “passive” mechanism (25).

This failure of the initial passive model has led to the development of many other hypothetical mechanisms and associated mathematical models for urine concentration in the IM. Wexler and colleagues explored, without significant

success, hypothetical mathematical models representing increasing structural complexity, including three-dimensional considerations (26–29). Jen and Stephenson (30) demonstrated mathematically that it was theoretically possible for the accumulation of a newly produced or “external” osmolyte in the inner medullary interstitium or vasculature to function as a concentrating agent, and Thomas and his colleagues (31,32) suggested that lactate might serve such a function. However, such a mechanism is probably insufficient to account completely for the magnitude of the gradient found experimentally (31). Moreover, no experimental data support the production or addition of such an osmolyte in the IM. Schmidt-Nielsen (33) suggested that contractions of the pelvic wall could serve as a source of energy for the inner medullary concentrating process, and Knepper and his colleagues (34) postulated that hyaluronan could act as a transducer to convert the mechanical energy of the contractions into a concentrating effect. However, this conversion has not yet been shown to be thermodynamically feasible, and there are no experimental measurements of such a process in the IM.

We believe, whatever the specific mechanism by which the urine is concentrated in the IM, that it must take into account the three-dimensional interactions between the tubular and vascular elements within the IM. Although, as noted above, an earlier model failed to demonstrate the significance of the three-dimensional structure (28), this model was based on very limited knowledge of that structure. Therefore, during the past decade, we have worked to develop a detailed understanding of the three-dimensional relationships of the tubules and vessels in the IM of the rat kidney by analysis of digital reconstructions of these structures produced from 1- μ m serial sections (35–42). For this purpose, we labeled the various tubules and vessels with antibodies to structure- and segment-specific proteins, usually transporters or channels. We also measured the transepithelial water and urea permeabilities of specific segments of isolated, perfused thin limbs of the loops of Henle (15,43). We used the rat kidney in our work because of the enormous amount of physiologic data, including data on the concentrating mechanism, available for this species. However, our preliminary work and studies by others (44,45) suggest that the three-dimensional relationships in the mouse renal medulla are similar to those in the rat. Our preliminary studies also indicate that rodent and human inner medullary architecture share fundamental similarities. Finally, we have developed several mathematical models to determine how these physiologic and structural features might relate to the concentrating mechanism (24,46–48). In this paper, we briefly review some of the main findings and put them into context with regard to the concentrating mechanism.

Three-Dimensional Reconstructions of Thin Limbs of the Loops of Henle in the Rat Inner Medulla

Our reconstructions of the thin limbs of the loops of Henle extending into the IM (37) revealed unexpected structural and functional features. First, although previous studies had assumed that the DTLs in the IM were highly permeable to water, we found that the DTLs of those loops

that have their bends within the first millimeter of the IM (about 40% of the inner medullary loops) fail to express the constitutive water channel, aquaporin-1 (AQP1), throughout their length, and that the DTLs of the longer loops (the remaining 60% of the loops) express AQP1 only for the first 40% of their length; they fail to express it over the remaining 60% (Figures 2 and 3). Studies with isolated, perfused tubules show, as expected, that segments expressing AQP1 have very high osmotic water permeability, whereas segments failing to express AQP1 have little or no osmotic water permeability (15). Therefore, a substantial portion of each DTL in the IM is relatively impermeable to water.

Second, the chloride channel CIC-K1 (the rat homolog of human CIC-Ka) begins to be expressed abruptly in the AQP1-negative portion of each DTL about 150–200 μ m above the bend, thereby defining a prebend segment, and continues to be expressed throughout the ATL (Figure 2) (37). The expression of CIC-K1 throughout each ATL apparently reflects the extremely high chloride permeability found in this segment (16). We assume that the prebend segment has a similar high chloride permeability. AQP1 is not expressed in the prebend segments or ATLs, reflecting the complete lack of osmotic water permeability in the ATLs (15,16,42) and, we assume, in the prebend segments as well. Both the DTLs and the ATLs have now been shown to have very high permeabilities to urea, but there is no evidence of known urea transporters to account for these (43,49).

Three-Dimensional Lateral and Vertical Relationships of Tubules and Vessels in the Initial Two Thirds of the Rat Inner Medulla

The tubules and vessels within the initial two thirds of the IM are arranged in a highly organized three-dimensional pattern, which we believe is related to their function in the urine-concentrating process. Coalescing clusters of CDs form the organizing motif for the arrangement of both loops of Henle and vasa recta as they descend through the IM (Figure 3, right panel). DTLs and descending vasa recta (DVR) are arranged outside each CD cluster in the intercluster region between the clusters (Figures 2, 3A, and 4). They tend to lie about as far as possible from any CD in any cluster. This arrangement continues as tubules and blood vessels descend along the corticopapillary axis (Figure 3, B–D) (35,37,39,41,42). In contrast, the ATLs and ascending vasa recta (AVR) are arranged both inside (intracluster region) and outside (intercluster region) each CD cluster (Figures 2, 4, and 5A) (39,40). In addition, the prebend region of each DTL (which is the same as the ATL in terms of water and solute permeability) is always in the intracluster region (Figure 2) (36,50). About 50% of the AVR lie outside the CD clusters, and about 50% lie inside the clusters (41,42). About half of those AVR outside the clusters lie next to DVR in an arrangement that appears to facilitate counter-current exchange, thereby helping to maintain any osmotic gradient established. The other half of the AVR outside the clusters appear to be arranged to move water and solute toward the outer medulla.

The 50% of the AVR that lie in the intracluster region are arranged in a way that suggests that they have a function

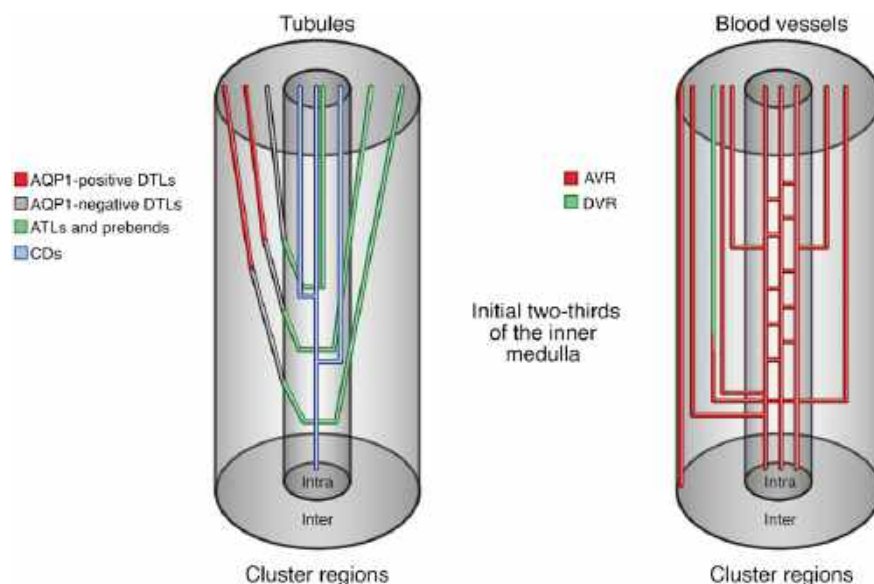


Figure 2. | Diagram of nephron and blood vessel architecture in the initial two thirds of the rat inner medulla. Collecting ducts (CDs) coalesce as they descend the corticopapillary axis, forming the intracluster region. DTLs and descending vasa recta (DVR) reside within the intercluster region. ATLs and ascending vasa recta (AVR) lie within the intercluster and intracluster regions. Modified from reference 22 with permission. AQP1, aquaporin-1.

quite different from that of the 50% that lie in the intercluster region (39,41,42). Almost all of these intracluster AVR closely abut a CD and lie parallel to it for a considerable distance along the corticopapillary axis (39). About four AVR on average are arranged symmetrically around each CD (Figures 4 and 6) so that about 55% of the surface of each CD is closely apposed to AVR. These AVR, in contrast to those in the intercluster region, have many small capillary branches connecting them to each other and sometimes to AVR in the intercluster region that lie closest to the intracluster region (Figure 2). This arrangement facilitates moving absorbate from the CDs in the cortical direction.

In the IM overall, there are about four times as many AVR as DVR (42). The excess AVR facilitate a vasa recta outflow from the IM that exceeds the inflow. This excess outflow returns the water absorbed from the IMCDs during the concentrating process to the cortex (42).

In the intracluster region, the arrangement of AVR, ATLs, and CDs, when viewed in a cross-section, form microdomains of interstitial space, or “interstitial nodal spaces” (Figures 4 and 6) (39). As shown, each of these spaces is bordered on one side by a CD (in which axial flow is toward the papilla tip), on the opposite side by one or more ATLs (in which axial flow is toward the outer medulla [OM]), and on the other two sides by AVR (in which flow is toward the OM). These spaces also appear to be bordered above and below by interstitial cells (51–53), making them discrete units about 1–10 μm thick. Therefore, these interstitial nodal spaces are probably arranged in stacks along the corticopapillary axis (Figure 6) (39). These confined spaces appear well configured for localized mixing of urea from the CDs with NaCl from the ATLs, and for the movement of these mixed and concentrated solutes to higher regions *via* the AVR (54) (Figure 4) (see below).

Three-Dimensional Relationships of Tubules and Vessels in the Terminal Third of the Rat Inner Medulla

The three-dimensional organization of the tubules and vessels differs substantially in the terminal third of the IM from that in the initial two thirds. In the terminal third of the IM, the organization around the CD clusters markedly diminishes as the CDs continue to coalesce. All the blood vessels show the same structural characteristics. Therefore, except for the vessels that closely abut CDs, which can clearly be identified as AVR, vessels with ascending flow cannot be differentiated from those with descending flow without direct measurements (38). Moreover, there is no clear arrangement of parallel vessels that would suggest countercurrent exchange (38). As CDs coalesce and increase in diameter, the number of AVR abutting them increases so that about 55% of the surface area of each CD continues to be closely apposed to AVR (38). Interstitial nodal spaces still appear to exist, but they decrease in number and increase in size as fewer and fewer loops reach into this region (38).

At the very tip of the papilla, CD clusters have disappeared as just a few remaining large CDs dominate the region and coalesce to form the terminal ducts of Bellini (38). The most striking feature in this region, however, is the arrangement of the bends of the loops of Henle. Throughout most of the length of the IM, descending loop segments form a U-shaped configuration, or hairpin bend, immediately before ascending toward the OM, but at the tip of the papilla only about half the loops have this hairpin bend (38). The remaining loops extend transversely, perpendicular to the corticopapillary axis, before ascending toward the OM. In some loops, this transverse extension literally wraps around the large CDs just before they merge with the papillary surface to form the terminal ducts of Bellini (38). These wide bends have 5- to 10-fold greater

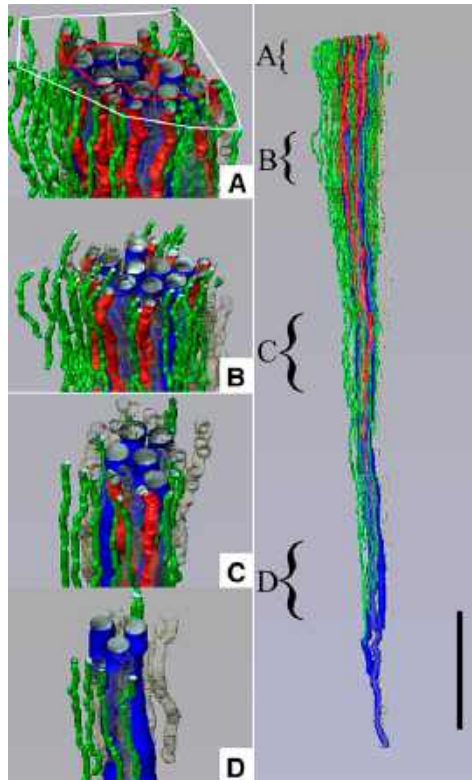


Figure 3. | Three-dimensional reconstruction showing spatial relationships of DVR (green tubules) and DTLs (red tubules) to CDs (blue tubules) for a single CD cluster. DTL segments that do not express AQP1 are shown in gray. DTLs and DVR lie at the periphery of the central core of CDs, within the intercluster region, and A–D show that this relationship continues along the entire axial length of the CD cluster. Axial positions of A–D are indicated by the curly brackets in the right panel. Tubules are oriented in a corticopapillary direction, with the upper edge of the image near the outer medullary–inner medullary border. The interstitial area within the red boundary line is the “intracluster” region, and the interstitial area between the red and white boundary lines is the “intercluster” region. Scale bar, 500 μm . Reproduced from reference 39 with permission.

transverse length than the hairpin bends and markedly increase the total loop-bend surface area relative to the CD surface area. This architecture could play a significant role in NaCl delivery and the development of the high osmolality at the papilla tip (55).

Mathematical Model Relating Structural and Permeability Findings to the Urine-Concentrating Process in the Inner Medulla

Over the past 10 years, we have developed a series of mathematical models of the urine-concentrating mechanism in the IM, which have incorporated the new information on the three-dimensional relationships of the vessels and tubules, the sites of transporters and channels, and the tubule permeabilities as this information became available (24,48,56–58). Here, we consider only the most recent version of these models (46).

This model has the following features: (1) the loop bends are distributed densely along the corticopapillary axis to

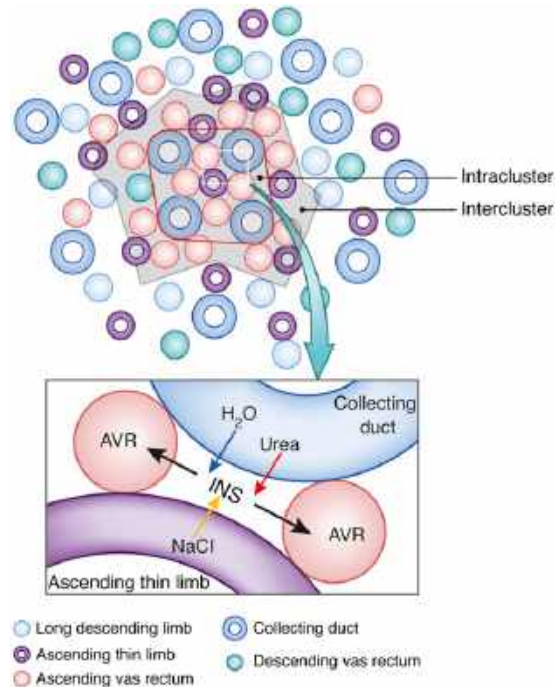


Figure 4. | Diagram of tubular organization in the rat renal medulla.

Upper: cross-section through the outer two thirds of the inner medulla, where tubules and vessels are organized around a collecting duct cluster. Lower: schematic configuration of a CD, AVR, an ATL, and an interstitial nodal space (INS). This illustrates the targeted delivery of NaCl from the ATL to the interstitial nodal space, where it can mix with urea and water from the CD. Modified from reference 48 with permission.

approximate loops turning back at all levels along this axis. (2) Interconnected regions represent the lateral and vertical relationships of tubules and vessels described above. (3) The high osmotic water permeability along the upper 40% of each DTL, the lack of osmotic water permeability along the lower 60% of each DTL and along the entire length of each ATL, the high NaCl permeability along each prebend and the complete length of each ATL, and the high urea permeability along the complete length of each DTL and ATL are represented (Figure 7). (4) The wide-bend loops at the tip of the papilla are also represented.

In the operation of the model, urea and water diffuse out of the CDs, as in the original Kokko and Rector (22) and Stephenson (23) model (Figure 7). Also, as in that model, this reduces the NaCl concentration in the interstitium, establishing a gradient for NaCl diffusion out of the loops of Henle (Figure 7). However, in contrast to the original model, this diffusion of NaCl occurs primarily out of the prebend regions and to a similar distance up the ATLs (Figure 7). The delivery of NaCl into the interstitium at the tip of the papilla occurs from the wide-bend loops over a very short axial distance. The diffusion of urea and water from the CDs and of NaCl from the prebends and ATLs occurs in the intracluster region, with the mixing of solutes occurring in the interstitial nodal spaces (Figure 4). This mixed and concentrated absorbate is then

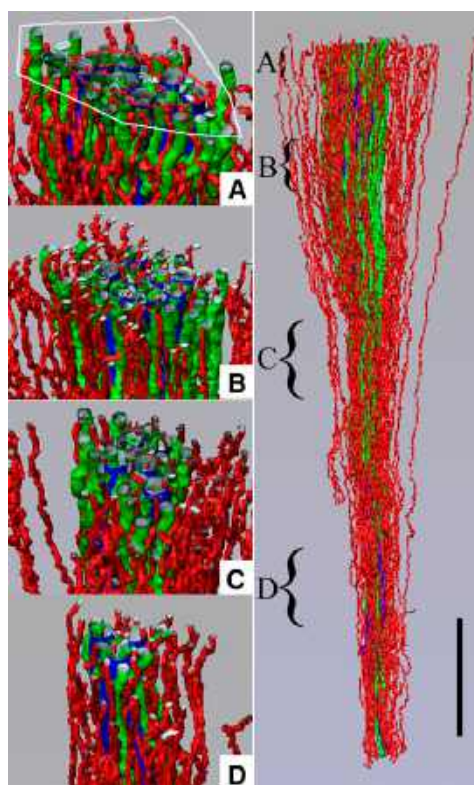


Figure 5. | Three-dimensional reconstruction showing spatial relationships of AVR (red tubules) and ATLs (green tubules) to CDs (blue tubules) for the same CD cluster shown in Figure 3. ATLs and AVR reside within both the intercluster and intracluster regions, and A–D show that this relationship continues along the entire axial length of the CD cluster. Axial positions of A–D are indicated by the curly brackets in the right panel. Tubules are oriented in a corticopapillary direction, with the upper edge of the image near the base of the inner medulla. The interstitial area within the red boundary line is the “intracluster” region and the interstitial area between the red and white boundary lines is the “intercluster” region. Scale bar, 500 μm . Reproduced from reference 39 with permission.

moved to less concentrated spaces at higher levels *via* the AVR bordering these spaces (Figure 4).

In contrast to the original model, there is no movement of NaCl or water into the lower 60% of each DTL (Figure 7). However, the high urea permeability of both thin limbs results in significant diffusion of urea into the DTLs and the lower part of the ATLs and out of the upper part of the ATLs so that the loops act as countercurrent exchangers (Figure 7).

This model predicts a urine osmolality (~ 1200 mOsmol/kg H_2O), Na^+ concentration, urea concentration, and flow rate in reasonable agreement with those measured in moderately antidiuretic rats (59) (Table 1). The model also predicts an osmolality at the tip of the longest loop in the IM similar to that in the urine and essentially equal to that measured experimentally (Table 1). In these respects, the model predictions are substantially better than the original passive model. However, this model, as long as it incorporates the high urea permeabilities measured in the thin limbs, does not predict the known

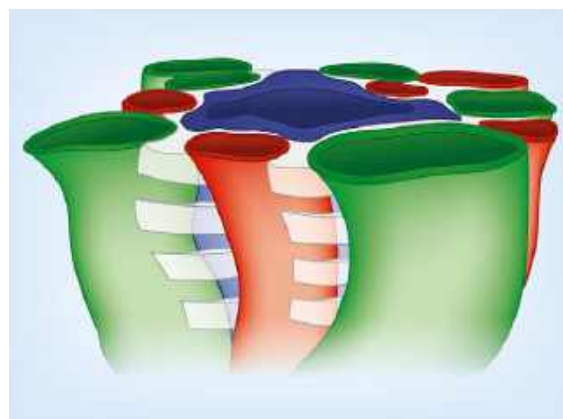


Figure 6. | Three-dimensional model illustrating stacks of interstitial nodal spaces (white) surrounding a single CD. Interstitial nodal spaces are separated by interstitial cells (not shown) with axial thickness of 1–10 μm . Green, ATL; red, AVR; blue, CD.

increasing axial Na^+ gradient along the loops. This leads to a predicted urea concentration higher than the Na^+ concentration at the tip of the longest loop, the opposite of the relationship measured experimentally (Table 1). Moreover, neither this model nor any other has yet been capable of generating a urine osmolality similar to that in a maximally antidiuretic rat (~ 2700 mOsmol/kg H_2O).

Summary

The urine-concentrating process in the mammalian kidney depends on the generation of an interstitial osmotic gradient from the corticomedullary border to the papilla tip, thereby providing the driving force for water absorption from the CDs during antidiuresis (Figure 1). In the OM, this osmotic gradient is generated by countercurrent multiplication within the loops of Henle, driven by the active transepithelial reabsorption of NaCl in the TALs (Figure 1). However, in the IM, the region in which the steepest osmotic gradient is generated, there are only ATLs, and there is no active transepithelial reabsorption of NaCl or any other solute by these structures (Figure 1). Thus, the mechanism by which the IM osmotic gradient is generated is a perplexing problem that has challenged renal physiologists for over half a century.

Early studies on inner medullary thin limb function in rats indicated that:

- DTLs are permeable to water.
- ATLs are impermeable to water, but highly permeable to NaCl.
- DTLs have low- and ATLs have moderate urea permeabilities.

Recent studies on inner medullary thin limb function, structure, and three-dimensional organization have modified these findings or added new ones as follows:

- Upper 40% of each DTL expresses AQP1 and is highly permeable to water.

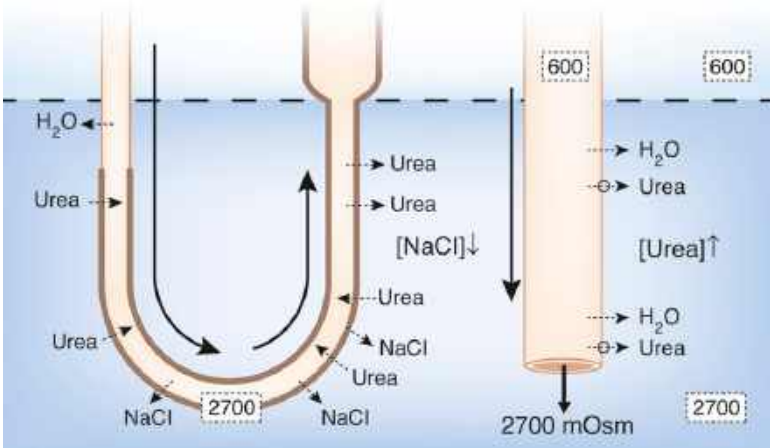


Figure 7. | Modification of Figure 1 to illustrate the unique permeabilities and solute fluxes of current solute-separation, solute-mixing passive model for concentrating urine. Thick tubule border indicates AQP1-null, water-impermeable segment of DTL as well as water-impermeable ATL and TAL. All arrows and symbols are as defined in Figure 1. The AQP1-null segment of the DTL is essentially impermeable to inorganic solutes and water. In this model, both the ATLs and the DTLs (including the AQP1-null segment) are highly permeable to urea. In contrast to the original passive model, passive NaCl reabsorption without water begins with the prebend segment and is most significant around the loop bend. Also, in contrast to previous models, urea moves passively into the entire DTL and early ATL, but as this urea-rich fluid further ascends in the ATL, it reaches regions of lower interstitial urea concentration and diffuses out of the ATL again. Thus, the loops act as countercurrent exchangers for urea.

Table 1. Comparison of model values and rat measurements			
Variable		Model ^a	Rat ^b
Urine			
Osmolality (mOsmol/kg H ₂ O)		1155	1216
Na ⁺ (mM)		254	100
Urea (mM)		554	345
Flow rate (μl/min)		3.58	2.27
Loop bend			
Osmolality (mOsmol/kg H ₂ O)		1235	1264
Na ⁺ (mM)		384	475
Urea (mM)		544	287
Model loop bend values are given for the longest loop of Henle.			
^a Data obtained from reference 46.			
^b Mean values or estimated mean values from reference 59.			

- Lower 60% of each DTL lacks AQP1 and is impermeable to water.
- Chloride channel CIC-K1 begins at DTL prebend segment and continues throughout ATL, accounting for high chloride permeability.
- Both DTLs and ATLs are highly permeable to urea.
- CD clusters form organizing motif for three-dimensional arrangement of thin limbs and vasa recta, with DTLs and DVR being outside the clusters and ATLs and AVR being both inside and outside the clusters.
- Arrangement of AVR, ATLs, and CDs within the clusters form interstitial nodal spaces bordered above and below by lateral interstitial cells, thereby creating microdomains for solute and water mixing.
- Loops turning at tip of papilla have wide lateral bends.

In an initial passive model for developing the IM osmotic gradient and concentrating the urine (22,23), urea, which

has been concentrated in the cortical CDs and outer medullary CDs by NaCl and water reabsorption, diffuses out of the IMCDs and draws water from the DTLs and IMCDs, thereby reducing the NaCl concentration in the IM interstitium and establishing a gradient for NaCl diffusion out of the ATLs to establish the interstitial osmotic gradient (Figure 1). However, this model failed to concentrate the urine when analyzed mathematically. In our most recent model (46), urea and water still diffuse out of the IMCDs, thereby reducing the interstitial NaCl concentration and establishing a gradient for NaCl diffusion out of the loops of Henle (Figure 7). However, with the new information available, this diffusion of NaCl occurs primarily out of the DTL prebend regions and to a similar distance up the ATLs (Figure 7). It also occurs from wide-bend loops over a very short axial distance at the tip of the papilla. NaCl from the loops and urea and water from the CDs are mixed and concentrated in the interstitial microdomains surrounding the CDs. Because the loops have very high urea permeabilities, they act as countercurrent exchangers for urea. When analyzed mathematically, this model now produces a urine concentration and tubule fluid concentration at the tip of the longest loops in good agreement with those measured in moderately antidiuretic rats. However, the model fails to produce a maximally concentrated urine or the appropriate interstitial axial Na⁺ gradient. Thus, it does not yet reflect the true physiologic operation of the urine concentrating mechanism in the IM.

Acknowledgments
Research in the authors' laboratories has been supported by the National Science Foundation, grant IOS-0952885 (T.L.P.), and by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases, grants DK08333 (T.L.P.), DK-89066 (A.T.L.) and DK-42091 (H.E.L.).

Disclosures

None.

References

- Hai MA, Thomas S: The time-course of changes in renal tissue composition during lysine vasopressin infusion in the rat. *Pflugers Arch* 310: 297–317, 1969
- Knepper MA: Measurement of osmolality in kidney slices using vapor pressure osmometry. *Kidney Int* 21: 653–655, 1982
- Wirz H, Hargitay B, Kuhn W: Lokalisation des Konzentrationsprozesses in der Niere durch direkte Kryoskopie. *Helv Physiol Acta* 9: 196–207, 1951
- Atherton JC, Green R, Thomas S: Influence of lysine-vasopressin dosage on the time course of changes in renal tissue and urinary composition in the conscious rat. *J Physiol* 213: 291–309, 1971
- Beck F, Dörge A, Rick R, Thürau K: Osmoregulation of renal papillary cells. *Pflugers Arch* 405[Suppl 1]: S28–S32, 1985
- Gottschalk CW, Mylle M: Micropuncture study of the mammalian urinary concentrating mechanism: Evidence for the countercurrent hypothesis. *Am J Physiol* 196: 927–936, 1959
- Hargitay B, Kuhn W: Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere. *Z Electrochem u ang Physikal Chem* 55: 539–558, 1951
- Kuhn W, Ramel A: Activer Salztransport als möglicher (und wahrscheinlicher) Einzeleffekt bei der Harnkonzentrierung in der Niere. *Helv Chim Acta* 42: 628–660, 1959
- Kuhn W, Ryffel K: Herstellung konzentrierter Lösungen aus verdünnten durch blosse Membranwirkung: ein Modellversuch zur Funktion der Niere. *Hoppe Seylers Z Physiol Chem* 276: 145–178, 1942
- Layton AT, Layton HE: Countercurrent multiplication may not explain the axial osmolality gradient in the outer medulla of the rat kidney. *Am J Physiol Renal Physiol* 301: F1047–F1056, 2011
- Burg MB, Green N: Function of the thick ascending limb of Henle's loop. *Am J Physiol* 224: 659–668, 1973
- Rocha AS, Kokko JP: Sodium chloride and water transport in the medullary thick ascending limb of Henle. Evidence for active chloride transport. *J Clin Invest* 52: 612–623, 1973
- Layton AT, Layton HE: A region-based mathematical model of the urine concentrating mechanism in the rat outer medulla. I. Formulation and base-case results. *Am J Physiol Renal Physiol* 289: F1346–F1366, 2005
- Chou C-L, Knepper MA: In vitro perfusion of chinchilla thin limb segments: Segmentation and osmotic water permeability. *Am J Physiol Renal. Fluid Electrolyte Physiol* 263: F417–F426, 1992
- Dantzler WH, Evans KK, Pannabecker TL: Osmotic water permeabilities in specific segments of rat inner medullary thin limbs of Henle's loops. *FASEB J* 23: 970–973, 2009
- Imai M, Kokko JP: Sodium chloride, urea, and water transport in the thin ascending limb of Henle. Generation of osmotic gradients by passive diffusion of solutes. *J Clin Invest* 53: 393–402, 1974
- Yool AJ, Brokl OH, Pannabecker TL, Dantzler WH, Stamer WD: Tetraethylammonium block of water flux in Aquaporin-1 channels expressed in kidney thin limbs of Henle's loop and a kidney-derived cell line. *BMC Physiol* 2: 4, 2002
- Imai M, Kusano E: Effects of arginine vasopressin on the thin ascending limb of Henle's loop of hamsters. *Am J Physiol* 243: F167–F172, 1982
- Marsh DJ, Azen SP: Mechanism of NaCl reabsorption by hamster thin ascending limbs of Henle's loop. *Am J Physiol* 228: 71–79, 1975
- Marsh DJ, Solomon S: Analysis of electrolyte movement in thin Henle's loops of hamster papilla. *Am J Physiol* 208: 1119–1128, 1965
- Pannabecker TL: Structure and function of the thin limbs of the loops of Henle. In: *Comprehensive Physiology*, edited by Terjung RL, Bethesda: John Wiley and Sons, 2012, pp 2063–2086
- Kokko JP, Rector FC Jr: Countercurrent multiplication system without active transport in inner medulla. *Kidney Int* 2: 214–223, 1972
- Stephenson JL: Concentration of urine in a central core model of the renal counterflow system. *Kidney Int* 2: 85–94, 1972
- Layton AT, Pannabecker TL, Dantzler WH, Layton HE: Two modes for concentrating urine in rat inner medulla. *Am J Physiol Renal Physiol* 287: F816–F839, 2004
- Sands JM, Layton HE: The urine concentrating mechanism and urea transporters. In: *The Kidney: Physiology and Pathophysiology*, edited by Alpern RJ, Hebert SC, Philadelphia: Elsevier, 2007, pp 1143–1177
- Wang X, Thomas SR, Wexler AS: Outer medullary anatomy and the urine concentrating mechanism. *Am J Physiol* 274: F413–F424, 1998
- Wang XQ, Wexler AS: The effects of collecting duct active NaCl reabsorption and inner medulla anatomy on renal concentrating mechanism. *Am J Physiol Renal* 270: F900–F911, 1996
- Wexler AS, Kalaba RE, Marsh DJ: Three-dimensional anatomy and renal concentrating mechanism. I. Modeling results. *Am J Physiol Renal* 260: F368–F383, 1991
- Wexler AS, Kalaba RE, Marsh DJ: Three-dimensional anatomy and renal concentrating mechanism. II. Sensitivity results. *Am J Physiol Renal* 260: F384–F394, 1991
- Jen JF, Stephenson JL: Externally driven countercurrent multiplication in a mathematical model of the urinary concentrating mechanism of the renal inner medulla. *Bull Math Biol* 56: 491–514, 1994
- Hervy S, Thomas SR: Inner medullary lactate production and urine-concentrating mechanism: A flat medullary model. *Am J Physiol Renal Physiol* 284: F65–F81, 2003
- Thomas SR: Inner medullary lactate production and accumulation: A vasa recta model. *Am J Physiol Renal Physiol* 279: F468–F481, 2000
- Schmidt-Nielsen B: August Krogh Lecture. The renal concentrating mechanism in insects and mammals: A new hypothesis involving hydrostatic pressures. *Am J Physiol* 268: R1087–R1100, 1995
- Knepper MA, Saidel GM, Hascall VC, Dwyer T: Concentration of solutes in the renal inner medulla: Interstitial hyaluronan as a mechano-osmotic transducer. *Am J Physiol Renal Physiol* 284: F433–F446, 2003
- Kim J, Pannabecker TL: Two-compartment model of inner medullary vasculature supports dual modes of vasopressin-regulated inner medullary blood flow. *Am J Physiol Renal Physiol* 299: F273–F279, 2010
- Pannabecker TL: Loop of Henle interaction with interstitial nodal spaces in the renal inner medulla. *Am J Physiol Renal Physiol* 295: F1744–F1751, 2008
- Pannabecker TL, Abbott DE, Dantzler WH: Three-dimensional functional reconstruction of inner medullary thin limbs of Henle's loop. *Am J Physiol Renal Physiol* 286: F38–F45, 2004
- Pannabecker TL, Dantzler WH: Three-dimensional architecture of collecting ducts, loops of Henle, and blood vessels in the renal papilla. *Am J Physiol Renal Physiol* 293: F696–F704, 2007
- Pannabecker TL, Dantzler WH: Three-dimensional architecture of inner medullary vasa recta. *Am J Physiol Renal Physiol* 290: F1355–F1366, 2006
- Pannabecker TL, Dantzler WH: Three-dimensional lateral and vertical relationships of inner medullary loops of Henle and collecting ducts. *Am J Physiol Renal Physiol* 287: F767–F774, 2004
- Pannabecker TL, Henderson CS, Dantzler WH: Quantitative analysis of functional reconstructions reveals lateral and axial zonation in the renal inner medulla. *Am J Physiol Renal Physiol* 294: F1306–F1314, 2008
- Yuan J, Pannabecker TL: Architecture of inner medullary descending and ascending vasa recta: Pathways for countercurrent exchange. *Am J Physiol Renal Physiol* 299: F265–F272, 2010
- Evans K, Pannabecker TL, Dantzler WH: Urea permeabilities in defined segments of rat renal inner medullary thin limbs of Henle's loops. *FASEB J* In press
- Zhai X-Y, Thomsen JS, Birn H, Kristoffersen IB, Andreassen A, Christensen EI: Three-dimensional reconstruction of the mouse nephron. *J Am Soc Nephrol* 17: 77–88, 2006
- Zhai XY, Birn H, Jensen KB, Thomsen JS, Andreassen A, Christensen EI: Digital three-dimensional reconstruction and ultrastructure of the mouse proximal tubule. *J Am Soc Nephrol* 14: 611–619, 2003
- Layton AT, Dantzler WH, Pannabecker TL: Urine concentrating mechanism: impact of vascular and tubular architecture and a proposed descending limb urea-Na⁺ cotransporter. *Am J Physiol Renal Physiol* 302: F591–F605, 2012

47. Layton AT, Gilbert RL, Pannabecker TL: Isolated interstitial nodal spaces may facilitate preferential solute and fluid mixing in the rat renal inner medulla. *Am J Physiol Renal Physiol* 302: F830–F839, 2012
48. Layton AT, Pannabecker TL, Dantzler WH, Layton HE: Functional implications of the three-dimensional architecture of the rat renal inner medulla. *Am J Physiol Renal Physiol* 298: F973–F987, 2010
49. Chou C-L, Knepper MA: In vitro perfusion of chinchilla thin limb segments: Urea and NaCl permeabilities. *Am J Physiol* 264: F337–F343, 1993
50. Westrick KY, Serack BJ, Dantzler WH, Pannabecker TL: Axial compartmentation of descending and ascending thin limbs of Henle's loops. *Am J Physiol Renal Physiol* 304: F308–F316, 2013
51. Kneen MM, Harkin DG, Walker LL, Alcorn D, Harris PJ: Imaging of renal medullary interstitial cells in situ by confocal fluorescence microscopy. *Anat Embryol (Berl)* 200: 117–121, 1999
52. Lemley KV, Kriz W: Anatomy of the renal interstitium. *Kidney Int* 39: 370–381, 1991
53. Takahashi-Iwanaga H: The three-dimensional cytoarchitecture of the interstitial tissue in the rat kidney. *Cell Tissue Res* 264: 269–281, 1991
54. Pannabecker TL, Dantzler WH, Layton HE, Layton AT: Role of three-dimensional architecture in the urine concentrating mechanism of the rat renal inner medulla. *Am J Physiol Renal Physiol* 295: F1271–F1285, 2008
55. Layton HE, Davies JM: Distributed solute and water reabsorption in a central core model of the renal medulla. *Math Biosci* 116: 169–196, 1993
56. Layton AT: A mathematical model of the urine concentrating mechanism in the rat renal medulla. I. Formulation and base-case results. *Am J Physiol Renal Physiol* 300: F356–F371, 2011
57. Layton AT: A mathematical model of the urine concentrating mechanism in the rat renal medulla. II. Functional implications of three-dimensional architecture. *Am J Physiol Renal Physiol* 300: F372–F384, 2011
58. Layton AT, Layton HE, Dantzler WH, Pannabecker TL: The mammalian urine concentrating mechanism: Hypotheses and uncertainties. *Physiology (Bethesda)* 24: 250–256, 2009
59. Pennell JP, Lacy FB, Jamison RL: An in vivo study of the concentrating process in the descending limb of Henle's loop. *Kidney Int* 5: 337–347, 1974

Published online ahead of print. Publication date available at www.cjasn.org.



Thick Ascending Limb of the Loop of Henle

David B. Mount

Abstract

The thick ascending limb occupies a central anatomic and functional position in human renal physiology, with critical roles in the defense of the extracellular fluid volume, the urinary concentrating mechanism, calcium and magnesium homeostasis, bicarbonate and ammonium homeostasis, and urinary protein composition. The last decade has witnessed tremendous progress in the understanding of the molecular physiology and pathophysiology of this nephron segment. These advances are the subject of this review, with emphasis on particularly recent developments.

Clin J Am Soc Nephrol 9: 1974–1986, 2014. doi: 10.2215/CJN.04480413

Introduction

The thick ascending limb (TAL) occupies a central anatomic and functional position in human renal physiology, with critical roles in the defense of the extracellular fluid volume, the urinary concentrating mechanism, calcium and magnesium homeostasis, bicarbonate and ammonium homeostasis, and urinary protein composition (1). The last decade has witnessed tremendous progress in the understanding of the molecular physiology and pathophysiology of this nephron segment. These advances are the subject of this review, with emphasis on particularly recent developments.

Anatomy and Morphology

The loop of Henle encompasses the thin descending limb, the thin ascending limb, and the TAL. Short-looped nephrons that originate from superficial and midcortical nephrons have a short descending limb within the inner stripe of the outer medulla; close to the hairpin turn of the loop, these tubules merge into the TAL (see Figure 1). By contrast, long-looped nephrons originating from juxtamedullary glomeruli have a long ascending thin limb. The TALs of long-looped nephrons begin at the boundary between the inner and outer medulla, whereas the TALs of short-looped nephrons may be entirely cortical. The ratio of medullary to cortical TAL for a given nephron is a function of the depth of its origin such that superficial nephrons primarily contain cortical TALs, whereas juxtamedullary nephrons primarily contain medullary TALs.

Aquaporin-1 expression is a marker of descending thin limbs and has been utilized to define the anatomy of the loops of Henle (2). The TAL begins abruptly after the thin ascending limb of long-looped nephrons and after an aquaporin-1-negative segment of short-limbed nephrons, immediately following the aquaporin-1-positive thin descending limb (2). The TAL then meets its parent glomerulus at the vascular pole; the plaque of renal tubular cells at this junction constitutes the macula densa, cells that share transport

characteristics with adjacent TAL cells. The distal convoluted tubule (DCT) begins at a variable distance after the macula densa, with an abrupt transition between cortical TAL cells expressing the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter (NKCC2; see Figure 2 and below) and DCT cells that express the thiazide-sensitive $\text{Na}^+\text{-Cl}^-$ cotransporter (NCC).

The TAL contains two morphologic subtypes: a rough-surfaced cell type (R cells) with prominent apical microvilli and a smooth-surfaced cell type (S cells) with an abundance of subapical vesicles (3,4) (see Figure 3). In the hamster TAL, cells can also be separated into those with high apical and low basolateral K^+ conductance and a weak basolateral Cl^- conductance (LBC cells), versus a second population with low apical and high basolateral K^+ conductance, with high basolateral Cl^- conductance (HBC cells) (3,5). The relative frequency of the morphologic and functional subtypes in the cortical and medullary TAL suggests that HBC cells correspond to S cells and LBC cells to R cells (3). Molecular characterization of this heterogeneity is still rudimentary; however, R and S cells clearly differ in the expression pattern of EGF (6) and NKCC2 (4). The functional correlates of this heterogeneity are discussed in the following sections.

Apical Transport of Na^+ , K^+ , and Cl^-

The TAL reabsorbs approximately 30% of filtered $\text{Na}^+\text{-Cl}^-$, with a steady drop in the luminal $\text{Na}^+\text{-Cl}^-$ concentration from approximately 140 mM in the inner stripe of the outer medulla to 30–60 mM at the macula densa (7). In addition to maintenance of the extracellular fluid volume and defense of arterial perfusion, $\text{Na}^+\text{-Cl}^-$ absorption by the TAL plays a pivotal role in the urinary concentrating mechanism. Specifically, active $\text{Na}^+\text{-Cl}^-$ absorption by the water-impermeable TAL dilutes the luminal fluid and drives the countercurrent multiplication that generates the axial osmolality gradient in the outer medulla, required for the vasopressin-dependent absorption of water by the collecting duct.

Renal Division,
Brigham and
Women's Hospital,
Veterans Affairs
Boston Healthcare
System, Boston,
Massachusetts

Correspondence:

Dr. David B. Mount,
Renal Division,
Brigham and
Women's Hospital,
Room 540, 4 Blackfan
Circle, Boston, MA
02115. Email:
dmount@partners.org

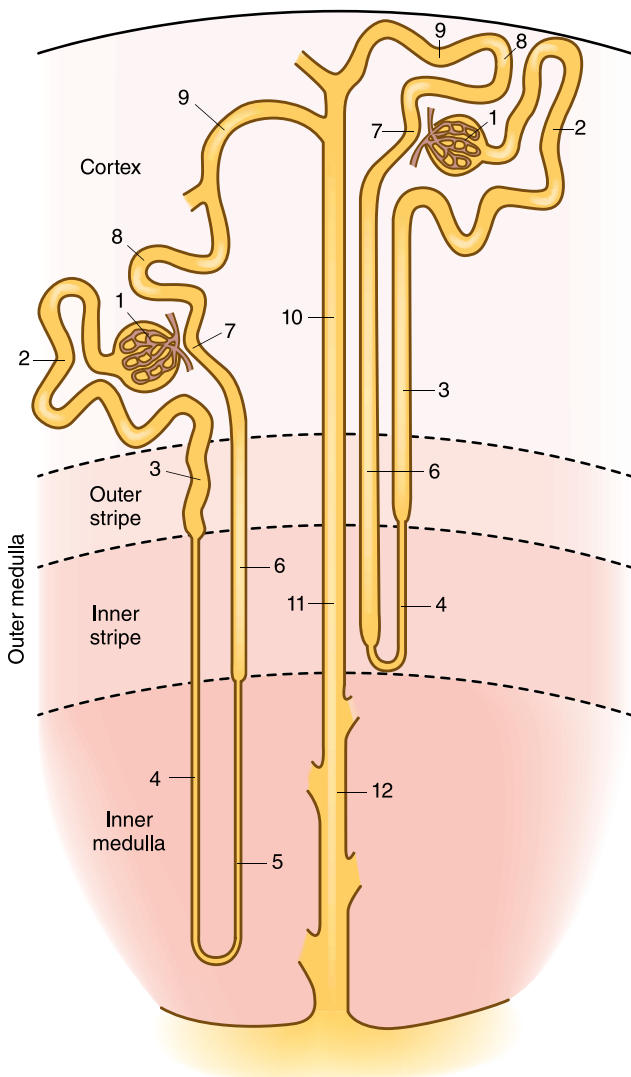


Figure 1. | Organization of the nephron, showing both short-looped and long-looped nephrons. See text for details relevant to the thick ascending limb (TAL). Within the cortex, a medullary ray is delineated by a dashed line. Structures are noted as follows: 1, glomerulus; 2, proximal convoluted tubule; 3, proximal straight tubule; 4, descending thin limb; 5, ascending thin limb; 6, TAL; 7, macula densa; 8, distal convoluted tubule; 9, connecting tubule; 10, cortical collecting duct; 11, outer medullary collecting duct; 12, inner medullary collecting duct.

The cells of the medullary TAL, cortical TAL, and macula densa share the same basic transport mechanisms (see Figure 2). Na^+ , K^+ , and Cl^- are cotransported across the apical membrane by NKCC2, an electroneutral $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter that is exquisitely sensitive to furosemide, a “loop” diuretic known for 4 decades to inhibit transepithelial Cl^- transport by the TAL (8). This transporter generally requires the simultaneous presence of all three ions such that the transport of Na^+ and Cl^- across the epithelium is mutually codependent and dependent on the luminal presence of K^+ (9). Functional expression of NKCC2 in *Xenopus laevis* oocytes yields Cl^- - and Na^+ -dependent uptake of $^{86}\text{Rb}^+$ (a radioactive substitute for K^+) and Cl^- - and K^+ -dependent

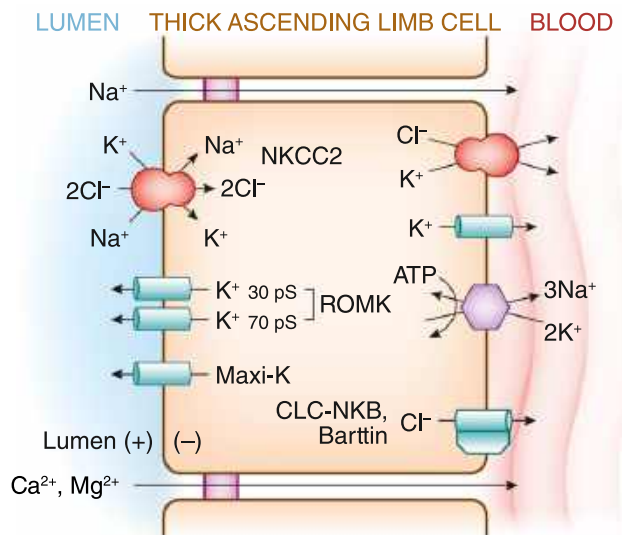


Figure 2. | Transepithelial $\text{Na}^+\text{-Cl}^-$ transport pathways in the TAL. See text for details. Barttin, Cl^- channel subunit; CLC-NKB, human Cl^- channel; KCC4, $\text{K}^+\text{-Cl}^-$ cotransporter-4; Maxi-K, calcium-activated maxi K^+ channel (also known as the BK channel); NKCC2, $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter-2; ROMK, renal outer medullary K^+ channel.

uptake of $^{22}\text{Na}^+$ (9–11), sensitive to micromolar concentrations of furosemide, bumetanide, and other loop diuretics (9).

NKCC2 is expressed along the entire TAL, in both R and S cells (4) (see Figure 3). NKCC2 expression in subapical vesicles is particularly prominent in smooth cells (4), consistent with the evolving understanding of vesicular trafficking in the regulation of NKCC2 (7). NKCC2 is also expressed in macula densa cells (4) (Figure 1), which are known to demonstrate apical $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport activity (12). Luminal loop diuretics applied at the macula densa block both tubuloglomerular feedback (13) and the suppression of renin release by luminal Cl^- (14), indicating that NKCC2 in the macula densa functions as the tubular sensor for both processes. The ability of loop diuretics to block tubuloglomerular feedback has been linked to their greater renal functional tolerance versus thiazides in advanced CKD (15). In contrast with the inhibitory effect of loop diuretics, volume depletion induced by thiazides augments the tubuloglomerular feedback response (16), causing a sharp drop in GFR in patients with CKD (15).

Alternative splicing of exon 4 of solute carrier family 12, member 1 (*SLC12A1*, the gene encoding NKCC2) yields NKCC2 proteins that differ in primary sequence within transmembrane domain 2 and the adjacent intracellular loop. There are thus three different variants of exon 4, denoted A, B, and F; the variable inclusion of these cassette exons yields distinct NKCC2-A, NKCC2-B, and NKCC2-F isoforms (9,11). Kinetic transporter characterization reveals that these isoforms differ dramatically in ion affinities (9,11). In particular, NKCC2-F has a very low affinity for Cl^- (K_m of 113 mM) and NKCC2-B has a very high affinity (K_m of 8.9 mM); NKCC2-A has an intermediate Cl^- affinity (K_m of 44.7 mM) (11). These isoforms differ in axial distribution along the tubule, with the F cassette expressed in the inner stripe of the outer medulla, the A cassette in the outer stripe, and the B cassette in cortical

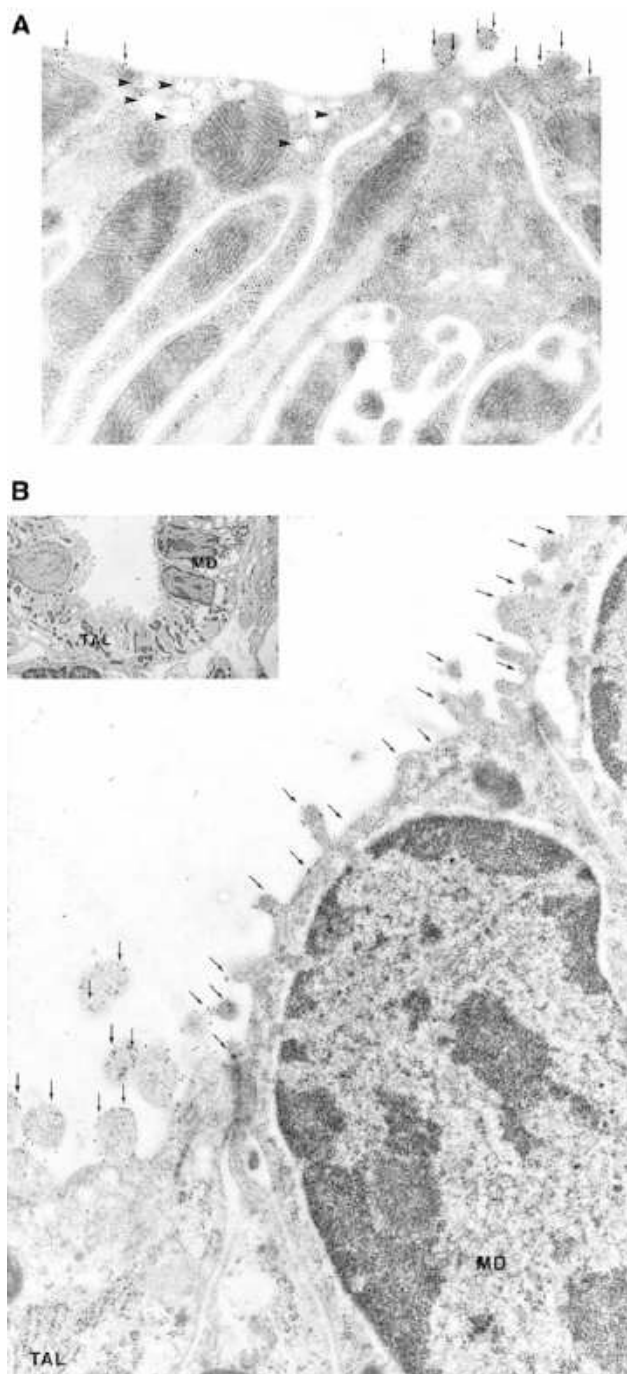


Figure 3. | Ultrastructural localization of NKCC2 protein in the TAL and macula densa (MD). (A) Immunoelectron microscopy of NKCC2 in the TAL. NKCC2 labeling is associated with apical plasma membrane (arrows) and small intracellular vesicles (arrowheads) of TAL cells. Both smooth-surfaced cells (left) and rough-surfaced cells (right) are labeled, with greater labeling of intracellular vesicles in smooth-surfaced cells. (B) Immunogold localization of NKCC2 in MD. Abundant NKCC2 labeling is associated with apical plasma membrane (arrows) of MD cells and TAL cells. Inset: overview showing MD cells and TAL cells. Regions indicated by MD are shown at higher power in main panel from adjacent section. Original magnification, $\times 3500$ in B; $\times 44,000$ in B inset. Reprinted from reference 4, with permission.

TAL (17). There is thus an axial distribution of the anion affinity of NKCC2 along the TAL, from a low-affinity, high-capacity transporter (NKCC2-F) in the inner stripe of the outer medulla to a high-affinity, low-capacity transporter (NKCC2-B) in the cortical TAL. This arrangement fits with the need for a progressive increase in the Cl^- affinity of the transporter as the luminal Cl^- concentration drops along the length of the TAL.

Microperfused TALs develop a lumen-positive potential difference when the tubular solutions contain $\text{Na}^+\text{-Cl}^-$ (18). This lumen-positive potential difference plays a critical role in physiology of the TAL, driving the paracellular transport of Na^+ , Ca^{2+} , and Mg^{2+} (see Figure 2). The conductivity of the apical membrane of TAL cells is predominantly K^+ selective; luminal recycling of K^+ *via* $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport and apical K^+ channels, along with basolateral depolarization due to Cl^- exit through Cl^- channels, generates the lumen-positive transepithelial potential difference (19,20).

Apical K^+ channels are critical for transepithelial $\text{Na}^+\text{-Cl}^-$ transport by the TAL. In microperfusion studies, the combined removal of K^+ from luminal perfusate and pharmacological blockade of apical K^+ channels results in a marked decrease in $\text{Na}^+\text{-Cl}^-$ reabsorption (21). Apical K^+ channels are thus required for sustained functioning of NKCC2; the low luminal concentration of K^+ in this nephron segment would otherwise become limiting for transepithelial $\text{Na}^+\text{-Cl}^-$ transport. The net transport of K^+ across perfused TAL epithelium is $<10\%$ that of Na^+ and Cl^- (22); approximately 90% of the K^+ transported by NKCC2 is recycled across the apical membrane *via* K^+ channels, resulting in minimal net K^+ absorption by the TAL (20).

Three subtypes of apical K^+ channels have been identified in the TAL, with differing unitary conductance characteristics: a 30-picosiemens (pS) channel, a 70-pS channel, and a high-conductance, calcium-activated maxi K^+ channel (also known as the big K^+ or BK channel) (23–25) (see Figure 2). The 70-pS channel mediates approximately 80% of the apical K^+ conductance of TAL cells (26). The low-conductance 30-pS channel shares several biophysical and regulatory characteristics with the cloned renal outer medullary K^+ channel (ROMK; otherwise known as $\text{K}_{\text{IR}}1.1$ or KCNJ1), the cardinal inward-rectifying K^+ channel that was initially cloned from rat renal outer medulla (27). ROMK protein has been identified at the apical membrane of medullary TAL, cortical TAL, and macula densa (28). The 30-pS channel is completely absent from the apical membrane of mice with homozygous deletion of the gene encoding ROMK (29), providing genetic evidence that ROMK mediates this 30-pS conductance. Notably, not all cells in the TAL are labeled with ROMK antibody (28), suggesting that ROMK might be absent in the HBC cells with high basolateral Cl^- conductance and low apical/high basolateral K^+ conductance (also see above) (3,5). HBC cells are thought to correspond to the smooth-surfaced morphologic subtype of TAL cells (S cells) (3); however, the relative expression of ROMK protein by immunoelectron microscopy in R and S cells has not yet been published. Regardless, the heterogeneity of ROMK expression indicates that apical K^+ recycling is not present in all epithelial cells within the TAL.

ROMK plays a critical role in $\text{Na}^+\text{-Cl}^-$ absorption by the TAL, given that loss-of-function mutations in the gene

encoding this channel are associated with Bartter's syndrome (30) (see Table 1). This genetic phenotype was initially discordant with the data suggesting that the higher-conductance 70-pS K^+ channel is the dominant channel at the apical membrane of TAL cells (26). This paradox was resolved by the observation that the 70-pS channel is also absent from the TAL of ROMK knockout mice, indicating that ROMK proteins form a subunit of the 70-pS channel (31). ROMK activity in the TAL is clearly modulated by associations with other proteins such that coassociation with other subunits to generate the 70-pS channel is perfectly compatible with the known physiology of this protein. ROMK thus associates with scaffolding proteins Na^+/H^+ exchanger regulatory factor (NHERF)-1 and NHERF-2, *via* the C-terminal PDZ binding motif of ROMK; NHERF-2 is coexpressed with ROMK in the TAL (32). The association of ROMK with NHERFs serves to bring ROMK into closer proximity to the cystic fibrosis transmembrane regulator protein (CFTR) (32). This ROMK-CFTR interaction is in turn required for the native ATP and glybenclamide sensitivity of apical K^+ channels in the TAL (33). Impaired CFTR-dependent regulation of ROMK in the TAL may potentially explain the propensity for hypochloremic alkalosis and "pseudo-Bartter's syndrome" in patients with cystic fibrosis (33,34).

Basolateral Transport

The basolateral Na^+/K^+ -ATPase is the primary exit pathway for Na^+ at the basolateral membrane of TAL cells. The Na^+ gradient generated by this Na^+/K^+ -ATPase activity also drives the apical entry of Na^+ , K^+ , and Cl^- *via* NKCC2, the furosemide-sensitive $Na^+-K^+-2Cl^-$ cotransporter (20). Inhibition of Na^+/K^+ -ATPase with ouabain thus collapses the lumen-positive potential difference and abolishes transepithelial Na^+-Cl^- transport in the TAL (35). The basolateral exit of Cl^- from TAL cells is primarily but not exclusively (36) electrogenic, mediated primarily by Cl^- channels (19,20). Intracellular Cl^- activity during transepithelial Na^+-Cl^- transport is above its electrochemical equilibrium (37), with an intracellular-negative voltage of

–40 to –70 mV that drives basolateral Cl^- exit (19,20). Reductions in basolateral Cl^- depolarize the basolateral membrane, whereas increases in intracellular Cl^- induced by luminal furosemide have a hyperpolarizing effect (37).

At least two CLC chloride channels, CLC-K1 and CLC-K2 (denoted CLC-NKA and CLC-NKB in humans), are coexpressed in the TAL (38). Several lines of evidence indicate that the dominant Cl^- channel in the TAL is encoded by CLC-K2/CLC-NKB. First, CLC-K1 is expressed at both apical and basolateral membranes of the thin ascending limb and the phenotype of CLC-K1 knockout mice is more consistent with primary dysfunction of thin ascending limbs (39), rather than the TAL. CLC-K2 protein, in turn, is heavily expressed at the basolateral membrane of the TAL, with additional expression in the DCT, connecting tubule, and α -intercalated cells (40). Second, loss-of-function mutations in CLC-NKB are associated with Bartter's syndrome (41), genetic evidence for a dominant role of this channel in Na^+-Cl^- transport by the human TAL. Finally, an *in vivo* study using whole-cell recording techniques suggests that CLC-K2 is the dominant Cl^- channel in TAL (42).

A key advance in the physiology of the TAL was the characterization of the "Barttin" subunit of CLC-K channels, which is coexpressed with CLC-K1 and CLC-K2 in several nephron segments, including the TAL (38). The human CLC-NKA and CLC-NKB paralogs are not functional in the absence of Barttin coexpression (43); hence, the full functional characterization of these channels depended on the discovery of Barttin. CLC-NKB coexpressed with Barttin is highly selective for Cl^- , with a permeability series of $Cl^- \gg Br^- = NO_3^- > I^-$ (38,43). Strikingly, despite the considerable homology between the CLC-NKA/NKB proteins, these channels differ considerably in pharmacologic sensitivity to various Cl^- channel blockers (44). This pharmacologic divergence suggests that the possibility that novel inhibitors specific for CLC-NKB could eventually be developed; such inhibitors would be expected to function as novel loop diuretics that would not require tubular excretion for natriuretic effects, acting instead at the basolateral membrane.

Table 1. Genetic classification of Bartter's syndrome

Subtype	Protein	Function	Phenotype	Comments/Variants
I	NKCC2	$Na-K-2Cl$ cotransporter	Antenatal BS	Variant presentations (<i>e.g.</i> , acidosis, normokalemia)
II	ROMK	K^+ channel	Antenatal BS	Transient neonatal hyperkalemia
III	CLC-NKB	Cl^- channel	Classic BS	No nephrocalcinosis Gitelman's syndrome in some patients Deafness with CLC-NKA loss
IV	Barttin	Cl^- channel subunit	Antenatal BS with deafness	No nephrocalcinosis Hypomagnesemia Renal failure
V	CaSR	Calcium receptor	BS-like	Hypocalcemia, autosomal dominant

See the text for details. NKCC2, $Na^+-K^+-2Cl^-$ cotransporter-2; BS, Bartter's syndrome; ROMK, renal outer medullary K^+ channel; CLC-NKB, human Cl^- channel; Barttin, Cl^- channel subunit; CaSR, calcium-sensing receptor.

Electroneutral K^+-Cl^- cotransport (see Figure 2) mediates K^+ -dependent Cl^- exit at the TAL basolateral membrane (37). The K^+-Cl^- cotransporter KCC4 is expressed at the basolateral membrane of medullary and cortical TAL, in addition to macula densa. To account for the effects on transmembrane potential difference of basolateral barium and/or increased K^+ , it was previously suggested that the basolateral membrane of the TAL contains a barium-sensitive K^+-Cl^- transporter (37,45); this is consistent with the barium sensitivity of KCC4 (46). Increases in basolateral K^+ cause Cl^- -dependent cell swelling in the *Amphiuma* early distal tubule, an analog of the mammalian TAL. In *Amphiuma* LBC cells with low basolateral conductance, analogous to mammalian LBC cells (3,5), this cell swelling was not accompanied by changes in basolateral membrane voltage or resistance (47), consistent with electroneutral K^+-Cl^- transport. By extension, KCC4 may play an important role in the basolateral chloride transport of LBC cells.

Epithelial cells within the TAL are influenced by changes in interstitial osmolality, swelling under hypotonic conditions, and shrinking under hypertonic conditions. Notably, however, the apical membrane of the TAL is completely water impermeable and TAL segments have an extremely low transepithelial water permeability. The basolateral membrane, however, expresses abundant Aquaporin-1 water channel protein, allowing for water flux across the basolateral membrane and changes in cell volume in response to changes in interstitial osmolality (48).

Paracellular Transport

The transport stoichiometry of NKCC2 ($1Na^+/1K^+/2Cl^-$) is such that additional transport mechanisms are necessary to balance the transport of Na^+ with the exit of double the amount of Cl^- at the basolateral membrane; this additional Na^+ is transported across the epithelium *via* the paracellular pathway (49,50) (see Figure 2). The ratio of net Cl^- transepithelial absorption to net Na^+ absorption through the paracellular pathway is thus 2.4 ± 0.3 in microperfused mouse medullary TAL segments (50), close to the ratio of 2.0 expected if 50% of Na^+ transport occurs *via* the paracellular pathway. The combination of a cation-permeable paracellular pathway and an "active transport" lumen-positive potential difference (19), generated indirectly by the basolateral $Na^+/K^+-ATPase$ (35), results in a doubling of active Na^+-Cl^- transport for a given level of oxygen consumption (49).

Tight junctions in the TAL are cation selective, with relative permeability of Na^+ to that of Cl^- (P_{Na}/P_{Cl}) of 2–5 (19,50). The reported transepithelial resistance in the TAL is between 10 and 50 $\Omega \text{ cm}^2$; although this resistance is higher than that of the proximal tubule, the TAL is not considered a "tight" epithelium. Notably, however, water permeability of the TAL is extremely low, <1% that of the proximal tubule (19). These "hybrid" characteristics—relatively low resistance and very low water permeability—allow the TAL to generate and sustain Na^+-Cl^- gradients of up to 120 mM (19).

The tight junctions of epithelia function as charge- and size-selective "paracellular channels," physiologic characteristics that are conferred by integral membrane proteins that cluster together at the tight junction; changes in the expression of these proteins can have marked effects on permeability, without affecting the number of junctional

strands (51). In particular, the charge and size selectivity of tight junctions is conferred in large part by the claudins, a large (>20) gene family of tetraspan transmembrane proteins. Mouse TAL cells coexpress claudin-3, claudin-10, claudin-11, claudin-14, claudin-16, and claudin-19 (52–54). Notably, the expression of claudin-19 in TAL cells is heterogeneous (53), analogous perhaps to the heterogeneity of ROMK expression (see above).

The TAL reabsorbs approximately 50%–60% of filtered magnesium and approximately 20% of filtered calcium, exclusively *via* the paracellular pathway. Mutations in human claudin-16 (paracellin-1) and claudin-19 (52) are associated with hereditary hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), genetic evidence that these claudins are critical for the cation selectivity of TAL tight junctions. Heterologous expression of claudin-16 (paracellin-1) in the anion-selective LLC-PK1 cell line increases Na^+ permeability, without affecting Cl^- permeability (55). Claudin-19 in turn reduces P_{Cl} in LLC-PK1 cells, without affecting cation permeability (56). The claudin-16 and claudin-19 proteins physically interact (56,57) and coexpression of claudin-16 and claudin-19 synergistically increases the P_{Na}/P_{Cl} ratio in LLC-PK1 cells (56). Knockdown of claudin-16 in transgenic mice increases Na^+ absorption in the downstream collecting duct, with development of hypovolemic hyponatremia after treatment with amiloride (58). Claudin-19 knockdown mice exhibit an increase in fractional excretion of Na^+ and a doubling in serum aldosterone (57). Both strains exhibit hypermagnesuria and hypercalciuria, with hypomagnesemia, replicating the human FHHNC phenotype. In summary, claudin-16 and claudin-19 are critical for the cation selectivity of tight junctions in the TAL, contributing significantly to the transepithelial absorption of Na^+ , Ca^{2+} , and Mg^{2+} in this nephron segment.

Other claudins expressed in the TAL either modulate the function of claudin-16/claudin-19 heterodimers or have independent effects on paracellular transport. Claudin-14 interacts with claudin-16, disrupting cation selectivity of the paracellular barrier in cells that also coexpress claudin-19 (59). Claudin-14 expression in the TAL is calcium dependent, *via* the calcium-sensing receptor (CaSR), providing a novel axis for calcium-dependent regulation of paracellular calcium transport (see below) (59–61). Claudin-10 in turn appears to specifically modulate paracellular Na^+ permeability, with impaired paracellular Na^+ transport but enhanced paracellular Ca^{2+} and Mg^{2+} in claudin-10 knockout mice (54).

Transport of NH_4^+ and HCO_3^-

The TAL also plays an important role in acid-base physiology, functioning in both renal bicarbonate reabsorption and ammonium (NH_4^+) excretion. Approximately 15% of filtered bicarbonate is reabsorbed by the TAL. Apical, carbonic anhydrase-dependent (62) bicarbonate reabsorption is accomplished by Na^+/H^+ exchange, primarily mediated by the Na^+/H^+ exchanger NHE3 (63) (see Figure 4). The apical exit of an H^+ ion is accompanied by basolateral exit of HCO_3^- (*i.e.*, bicarbonate absorption). There are several basolateral exit mechanisms for bicarbonate in the TAL (64), including Cl^-/HCO_3^- exchange, $K^+-HCO_3^-$ cotransport (likely mediated by the K^+-Cl^- cotransporter KCC4), and Na^+/H^+ exchange. A basolateral $Na^+-HCO_3^-$ cotransporter (NBCn1) is heavily expressed in the TAL but is thought to primarily

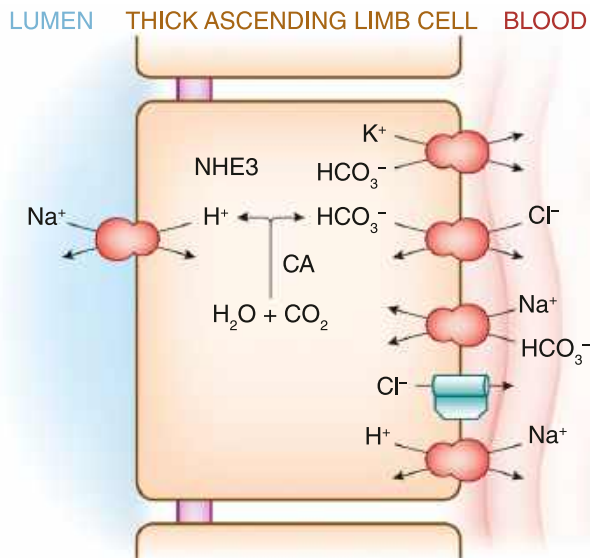


Figure 4. | Bicarbonate transport pathways within the TAL. See text for details. NHE3, Na^+/H^+ exchanger-3; CA, carbonic anhydrase.

function in bicarbonate entry at the basolateral membrane, rather than exit (65). Bicarbonate reabsorption by the TAL is regulated by acid-base status, and is upregulated in acidosis and downregulated in metabolic alkalosis (66).

Ammonium is generated by the proximal tubule in response to metabolic acidosis, reabsorbed by the TAL (67), and is concentrated by countercurrent multiplication within the medullary interstitium (67,68), from whence it is transported down its concentration gradient *via* apical NH_3 carriers in the collecting duct (69). The NH_4^+ ion has the same ionic radius as K^+ and can be transported by NKCC2 (70) and other K^+ transporters (see Figure 5). NH_4^+ transport *via* apical K^+ channels and paracellular

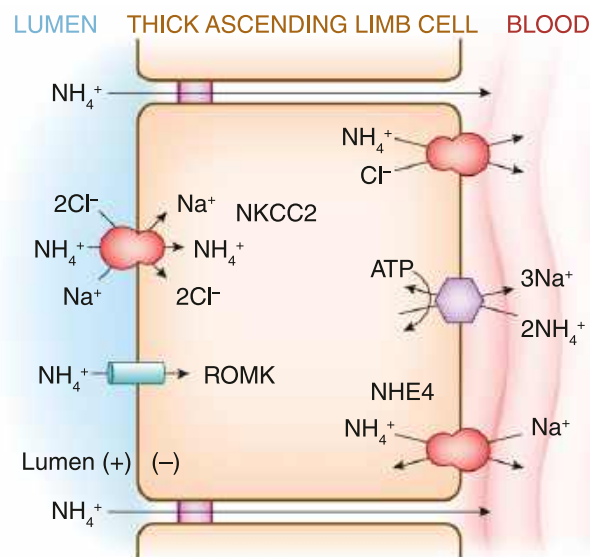


Figure 5. | Ammonium transport pathways within the TAL. See text for details. NH_4^+ , ammonium; NHE4, Na^+/H^+ exchanger-4.

transport play lesser roles under physiologic conditions (67). NH_4^+ exits the TAL predominantly *via* the basolateral Na^+/H^+ exchanger NHE4, functioning in $\text{Na}^+/\text{NH}_4^+$ exchange mode (71). The capacity of the TAL to reabsorb NH_4^+ and, as a result, the corticomedullary NH_4^+ gradient is increased during acidosis (67,68), due in part to an induction of NKCC2 (70) and NHE4 (71). Dysfunction or inhibition of the TAL, as in Bartter's syndrome or loop diuretic administration for example, typically causes metabolic alkalosis, somewhat obscuring the role of the TAL in acid and NH_4^+ homeostasis. Notably, however, pediatric patients with Bartter's syndrome due to NKCC2 deficiency can initially present with metabolic acidosis (72), perhaps because of a defect in medullary NH_4^+ accumulation.

Increasing the luminal K^+ concentration in perfused TAL markedly inhibits active NH_4^+ absorption, likely because of competition between K^+ and NH_4^+ for transport *via* NKCC2 (67). Hyperkalemia thus induces acidosis in rats by reducing NH_4^+ accumulation by the TAL, collapsing the NH_4^+ gradient between the vasa recta (surrogate for interstitial fluid) and collecting duct (73). Clinically, patients with hyperkalemic acidosis due to hyporeninemic hypoaldosteronism can demonstrate an increase in urinary NH_4^+ excretion in response to normalization of plasma K^+ with cation-exchange resins (74), indicating a significant role for hyperkalemia in generation of the acidosis. This physiology may gain broader relevance if and when novel potassium binders (75) become clinically available for chronic management of hyperkalemia.

Regulation of Ion Transport in the TAL

Activating Influences

Transepithelial Na^+/Cl^- transport by the TAL is regulated by multiple competing neurohumoral influences. In particular, increases in intracellular cAMP tonically stimulate ion transport in the TAL; the list of stimulatory hormones and mediators that increase cAMP in this nephron segment includes vasopressin, parathyroid hormone (PTH), glucagon, calcitonin, and β -adrenergic activation. These overlapping cAMP-dependent stimuli are thought to result in maximal baseline stimulation of transepithelial Na^+/Cl^- transport (76). This baseline activation is in turn modulated by a number of negative influences, most prominently prostaglandin E_2 (PGE_2) and extracellular Ca^{2+} . Other hormones and autoids working through cGMP-dependent signaling, including nitric oxide, also have potent negative effects on Na^+/Cl^- transport within the TAL (7). By contrast, angiotensin II has a stimulatory effect on Na^+/Cl^- transport within the TAL (77,78).

Vasopressin, acting through V2 receptors (79), is the most extensively studied positive modulator of transepithelial Na^+/Cl^- transport in the TAL. Vasopressin activates apical $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport within minutes in perfused mouse TAL segments, and also exerts longer-term influence on NKCC2 expression and function. The acute activation of apical $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport is achieved at least in part by the stimulated exocytosis of NKCC2 proteins, from subapical vesicles to the plasma membrane (7). Activation of NKCC2 is also associated with the phosphorylation of a cluster of N-terminal threonines in the transporter protein; treatment of rats with the V2 agonist desmopressin

(dDAVP; Sanofi-Aventis) induces phosphorylation of these residues *in vivo*, as measured with a phospho-specific antibody (80). These threonine residues are substrates for the homologous STE20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative stress-responsive kinase 1 (OSR1) kinases, initially identified by Gagnon *et al.* as key regulatory kinases for NKCC1 and other cation-chloride cotransporters (81). SPAK and OSR1, in turn, are activated by upstream WNK (with no lysine [K]) kinases.

The N-terminal phosphorylation of NKCC2 by OSR1 kinase appears to be critical for activity of the transporter in the native TAL. The N terminus of NKCC2 contains a predicted binding site for SPAK and OSR1 (82), proximal to the sites of regulatory phosphorylation; the analogous binding site is required for activation of the NKCC1 cotransporter (83). SPAK and OSR1 also require the sorting protein-related receptor with A-type repeats 1 (SORL1) (see also Figure 6) for proper trafficking within TAL cells such that targeted deletion of SORL1 results marked reduction in N-terminal NKCC2 phosphorylation (84). Of the two kinases, OSR1 is evidently more critical for NKCC2 function in the TAL, given the loss of function of the TAL with reduced N-terminal NKCC2 phosphoprotein in mice with targeted TAL-specific deletion of OSR1 (85).

The role of the upstream WNK kinases is illustrated by the phenotype of a “knock-in” mouse strain in which mutant SPAK or OSR1 cannot be activated by upstream WNK kinases (86); these mice have a marked reduction in N-terminal phosphorylation of both NKCC2 and the thiazide-sensitive NCC, with associated salt-sensitive hypotension. The upstream WNK kinases appear to regulate SPAK/OSR1 and NKCC2 in chloride-dependent fashion, phosphorylating and activating SPAK/OSR1 and the transporter in response to a reduction in intracellular chloride concentration (87). However, the adaptor protein calcium-binding protein 39 can dimerize and activate SPAK or OSR1 kinase monomers, bypassing the upstream phosphorylation by WNK kinases (88) (see also Figure 6).

Vasopressin has also been shown to alter the stoichiometry of furosemide-sensitive apical Cl^- transport in the TAL, from a K^+ -independent $\text{Na}^+\text{-Cl}^-$ mode to the classic $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport stoichiometry (49). Underscoring the metabolic advantages of paracellular Na^+ transport, which is critically dependent on the apical entry of K^+ *via* $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport (see above), vasopressin accomplishes a doubling of transepithelial $\text{Na}^+\text{-Cl}^-$ transport without affecting transcellular $\text{Na}^+\text{-Cl}^-$ transport. This doubling in transepithelial absorption occurs without an increase in O_2 consumption (49), highlighting the energy efficiency of ion transport by the TAL. The mechanism of this switch remains unknown. However, vasopressin induces cAMP-dependent phosphorylation of several serines and threonines in NKCC2 (89), potentially modulating K^+ dependence of the transporter.

In addition to its acute effects on NKCC2, vasopressin increases transepithelial $\text{Na}^+\text{-Cl}^-$ transport by activating apical K^+ channels and basolateral Cl^- channels in the TAL (76,90). Vasopressin also has considerable long-term effects on transepithelial $\text{Na}^+\text{-Cl}^-$ transport by the TAL. Sustained increases in circulating vasopressin result in marked hypertrophy of medullary TAL cells, accompanied by a doubling in baseline active $\text{Na}^+\text{-Cl}^-$ transport (90). Water restriction

or treatment with dDAVP also results in an increase in abundance of the NKCC2 protein in rat TAL cells. Consistent with a direct effect of vasopressin-dependent signaling, expression of NKCC2 is reduced in mice with a heterozygous deletion of the G_s stimulatory G protein, through which the V2 receptor activates cAMP generation (90).

Inhibitory Influences

The tonic stimulation of transepithelial $\text{Na}^+\text{-Cl}^-$ transport by cAMP-generating hormones is modulated by a number of negative neurohumoral influences. In particular, extracellular Ca^{2+} and PGE_2 exert potent inhibitory effects, through a plethora of synergistic mechanisms. Both extracellular Ca^{2+} and PGE_2 activate the G_i inhibitory G protein in TAL cells, opposing the stimulatory, G_s -dependent effects of vasopressin on intracellular levels of cAMP (91). Extracellular Ca^{2+} exerts its effect through the CaSR, which is heavily expressed at the basolateral membrane of TAL cells (91,92); PGE_2 primarily signals through EP_3 PG receptors (76). The increases in intracellular Ca^{2+} due to the activation of the CaSR and other receptors directly inhibits cAMP generation by a Ca^{2+} -inhibitable adenylate cyclase that is expressed in the TAL, accompanied by an increase in phosphodiesterase-dependent degradation of cAMP (91,93). Abrogation of the tonic negative effect of PGE_2 with indomethacin results in a considerable increase in abundance of the NKCC2 protein (90), whereas targeted deletion of the CaSR in mouse TAL activates NKCC2 *via* increased N-terminal phosphorylation (60).

Activation of the CaSR and other receptors in the TAL also results in the downstream generation of AA metabolites with potent negative effects on $\text{Na}^+\text{-Cl}^-$ transport. Extracellular Ca^{2+} thus activates phospholipase A_2 in TAL cells, leading to the liberation of AA. This AA is in turn metabolized by cytochrome P450 ω -hydroxylase to 20-hydroxyeicosatetraenoic acid, or by cyclooxygenase-2 to PGE_2 ; cytochrome P450 ω -hydroxylation generally predominates in response to activation of the CaSR in TAL (91). 20-Hydroxyeicosatetraenoic acid inhibits apical $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport, apical K^+ channels, basolateral Cl^- channels, and the basolateral $\text{Na}^+/\text{K}^+\text{-ATPase}$ (76,91,94).

The relative importance of the CaSR in the regulation of $\text{Na}^+\text{-Cl}^-$ transport by the TAL is dramatically illustrated by the phenotype of rare patients with gain-of-function mutations in this receptor. In addition to suppressed PTH and hypocalcemia, the usual phenotype caused by gain-of-function mutations in the CaSR (autosomal dominant hypoparathyroidism), these patients manifest a hypokalemic alkalosis, polyuria, and increases in circulating renin and aldosterone (95,96). Therefore, the persistent inhibition of $\text{Na}^+\text{-Cl}^-$ transport in the TAL by these overactive mutants of the CaSR causes a rare subtype of Bartter's syndrome, type V in the genetic classification of this disease (91) (see Table 1).

Activation of the CaSR also modulates the claudin repertoire of TAL cells, leading to PTH-independent hypercalciuria (59–61,92). This involves a novel feedback mechanism wherein activation of the CaSR downregulates two microRNAs (miR-9 and miR-374) that otherwise bind to the 3'-untranslated region of the claudin-14 mRNA and destabilize the transcript (59). This CaSR-dependent downregulation of these microRNAs leads to increased expression of claudin-14 protein, inhibition of

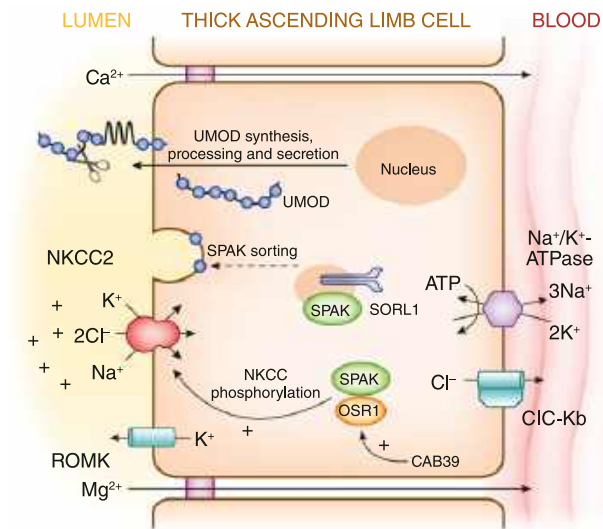


Figure 6. | Model for the interaction of ROMK, NKCC2, SORL1, SPAK/OSR1, and CAB39 with uromodulin in the regulation of ion transport in the TAL. CAB39, calcium-binding protein 39; OSR1, oxidative stress-responsive kinase 1; SORL1, sorting protein-related receptor with A-type repeats 1; SPAK, STE20/SPS1-related proline/alanine-rich kinase; Modified from reference 104, with permission.

claudin-16/claudin-19 heterodimers, reduced paracellular calcium permeability in the TAL, and hypercalciuria (59–61). This interaction provides a potential signaling pathway to explain the association between common variants in the human claudin-14 gene and hypercalciuric nephrolithiasis (97).

Uromodulin

TAL cells are unique in expressing the membrane-bound, glycosyl-phosphatidylinositol-anchored protein uromodulin (Tamm–Horsfall glycoprotein) (see Figure 7), which is not expressed by macula densa cells or the downstream DCT. Uromodulin can be released by proteolytic cleavage at the apical membrane and is secreted as the most abundant protein in normal human urine (20–100 mg/d) (1).

Uromodulin has a host of emerging roles in the physiology and biology of the TAL. A high-salt diet increases uromodulin expression (1), suggesting a role in ion transport. In this regard, uromodulin facilitates membrane trafficking and function of the NKCC2 protein (98), with similar effects on apical ROMK protein (99). Uromodulin also protects against nephrolithiasis, with the development of calcium oxalate stones in uromodulin knockout mice and evident protective alleles in humans (1). Potential mechanisms for this effect include reduced aggregation of nascent crystals (1) and activation of downstream transient receptor potential cation channel subfamily V member 5 epithelial calcium channels in the DCT by secreted uromodulin (100), with the development of hypercalciuria under uromodulin-deficient conditions. Other possible roles for uromodulin include a defensive role against urinary tract infection and possible roles in innate immunity (1). In disease, interactions between monoclonal free light chains and uromodulin are thought to be critical for cast

formation and AKI in cast nephropathy associated with multiple myeloma (101).

Autosomal dominant mutations in the *UMOD* gene encoding uromodulin are associated with medullary cystic disease type 2 and familial juvenile hyperuricemic nephropathy. Now collectively referred to as uromodulin-associated kidney disease (UAKD), this syndrome includes progressive tubulointerstitial damage and CKD, variably penetrant hyperuricemia and gout, and variably penetrant renal cysts that are typically confined to the corticomedullary junction (1). The causative mutations tend to affect conserved cysteine residues within the N-terminal half of the protein, leading to protein misfolding and retention within the endoplasmic reticulum (1,102) (see Figure 7).

Genome-wide association studies recently linked more common genetic variants in the *UMOD* promoter with the risk of CKD and hypertension (1). These susceptibility variants have a high frequency (approximately 0.8) and confer an approximately 20% higher risk for CKD and a 15% risk for hypertension (103). These polymorphisms are associated with more abundant renal uromodulin transcript and higher urinary uromodulin excretion (103,104), due to activating effects on the *UMOD* promoter (103). Overexpression of uromodulin in transgenic mice leads to distal tubular injury, with segmental dilation and increased tubular cast area relative to wild-type mice. Similar lesions were increased in frequency in older humans homozygous for susceptibility variants in *UMOD*, compared with those homozygous for protective variants (103). Uromodulin-transgenic mice also manifested salt-sensitive hypertension, owing to activation of the SPAK kinase and activating N-terminal phosphorylation of NKCC2. Again, human hypertensive individuals homozygous for susceptibility variants in *UMOD* appear to have an analogous phenotype, with exaggerated natriuresis in response to furosemide compared with those who are homozygous for protective variants (103). These findings are compatible with the stimulatory effects of uromodulin on the NKCC2 (98) and ROMK (99) transport proteins. Uromodulin excretion appears to parallel transport activity of the TAL and with common polymorphisms in the *KCNJ1* gene encoding ROMK and two genes involved in regulating SPAK/OSR1 kinase activity (*SORL1* and *CAB39*) (104). These latter genetic data link uromodulin function with the various signaling pathways that control $\text{Na}^+\text{-Cl}^-$ transport within the TAL (see Figure 6).

Pathophysiology of the TAL

It is no surprise that the TAL plays a significant role in the pathophysiology of disease, given its pivotal role in so many aspects of renal physiology. An understanding of TAL physiology leads to greater bedside appreciation of the mechanisms associated with its involvement in human pathophysiology. For example, as discussed above, hyperkalemia leads to an increase in tubular K^+ concentration within the TAL, competition between tubular K^+ and NH_4^+ for apical transport *via* NKCC2 (67), reduced trans-epithelial NH_4^+ transport, blunted countercurrent multiplication of interstitial NH_4^+ concentration, reduced urinary NH_4^+ excretion, and metabolic acidosis (73,74). Other associations between TAL dysfunction and human

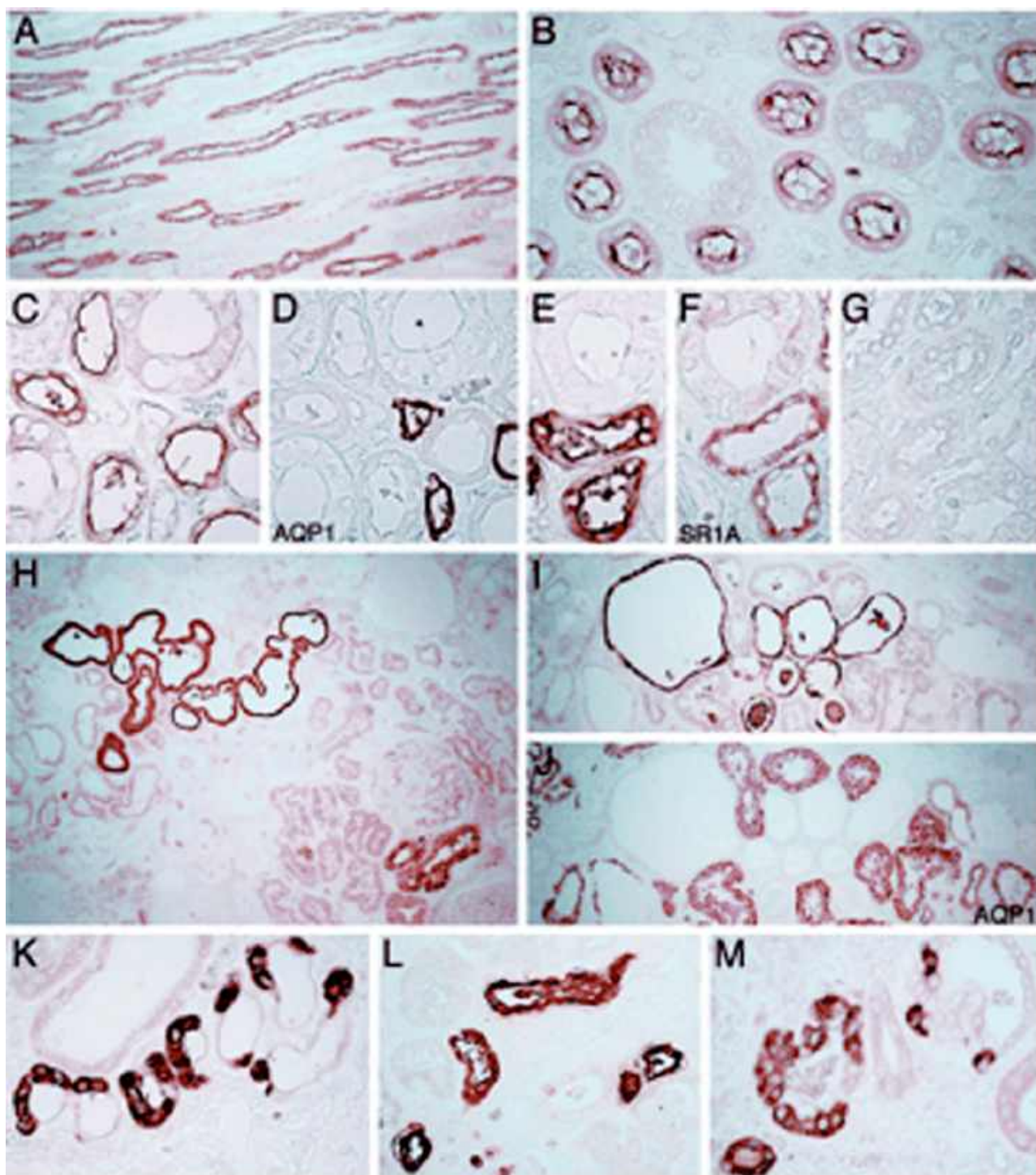


Figure 7. | Expression patterns and distribution of uromodulin in normal and diseased kidney. The segmental distribution and staining pattern of uromodulin was compared in normal kidneys (A–G), and in three kidneys with UAKD due to *UMOD* mutations (H–M). In the normal human kidney, uromodulin is distributed primarily in the TAL segments (A), with a distinct apical membrane reactivity (B). The segmental distribution to the TAL was demonstrated by lack of cross-reactivity with AQP1 (C and D) and codistribution with SR1A on serial sections (E and F). No specific staining was detected when using nonimmune IgG (G). (H–M) The expression and staining pattern for uromodulin was significantly modified in the three kidneys harboring *UMOD* mutations. Intense staining for uromodulin was detected in a subset of tubule profiles (H and I) that are sometimes enlarged or cystic. The tubule profiles stained for uromodulin are negative for AQP1 (I and J). (K–M) At higher magnification, the staining for uromodulin is intense, diffusely intracellular, and also heterogeneous within tubular cells. AQP1, aquaporin 1; SR1A, serotonin receptor 1A; UAKD, uromodulin-associated kidney disease. Modified from reference 102, with permission.

disease are more complex, such as the calcium-dependent regulation of claudin-10 and its genetic role in hypercalciuric nephrolithiasis (59–61) (see the discussion of paracellular transport).

Inhibition of TAL function with loop diuretics can also have predictable beneficial effects in specific renal syndromes. For example, by collapsing the lumen-positive potential difference and thus reducing paracellular calcium transport, loop diuretics—combined with adequate saline administration—can enhance calcium excretion in hypercalcemia (105). Loop diuretics combined with oral salt supplementation are also an effective chronic therapy for the syndrome of inappropriate antidiuretic hormone (106), blunting the countercurrent mechanism, increasing water excretion, and correcting the associated hyponatremia. An acquired dysfunction of NKCC2 after ureteral obstruction can in turn lead to the salt wasting and impaired urinary concentrating ability that is characteristic of postobstructive renal function (107).

Hereditary loss of function or dysfunction of TAL is discussed in the various sections above. Loss-of-function mutations in TAL $\text{Na}^+\text{-Cl}^-$ transport are associated with Bartter's syndrome, familial hypokalemic metabolic alkalosis. Patients with "classic" Bartter's syndrome typically suffer from polyuria and impaired urinary concentrating ability. They may have an increase in urinary Ca^{2+} excretion and approximately 20% are hypomagnesemic (108); other features include marked elevation of plasma angiotensin II, plasma aldosterone, and plasma renin. By contrast, patients with Gitelman's syndrome, caused by recessive loss of function mutations of NCC (the thiazide-sensitive $\text{Na}^+\text{-Cl}^-$ cotransporter in the DCT), are markedly hypocalciuric and universally hypomagnesemic. Patients with "antenatal" Bartter's syndrome present earlier in life with a severe systemic disorder characterized by marked electrolyte wasting, polyhydramnios, and significant hypercalciuria with nephrocalcinosis. Genetic classification of Bartter's syndrome is outlined in Table 1. Although there is significant phenotypic overlap and phenotypic variability, the various phenotypes are predictable in many respects from the underlying physiology of the genes involved. Other genetic causes of TAL dysfunction include UAKD and FHHNC; although the associated defects in TAL are less severe than in Bartter's syndrome, these disorders can also encompass polyuria and impaired concentrating ability, due to dysfunction in the countercurrent mechanism. Relative hypovolemia and the associated neurohumoral response can lead to hyperuricemia with or without gout, as has been reported in Bartter's syndrome (109), UAKD (1), and FHHNC (110).

Finally, a considerable body of evidence links hypertension with increased NKCC2 activity in both human hypertension and animal models of hypertension (7). Much of this association may be due to gain-of-function variants in the *UMOD* gene encoding uromodulin (1,103). These provocative findings highlight the role of the TAL in hypertension and may lead to a reappraisal of the therapeutic approach to hypertensive individuals with common, at-risk *UMOD* genotypes. Furthermore, the association of *UMOD* variation with an increased risk of CKD identifies uromodulin as a potential therapeutic target in both hypertension and CKD.

Acknowledgments

This review is dedicated to the memory of Steven C. Hebert, who made multiple seminal contributions to the physiology and pathophysiology of the TAL.

D.B.M. is supported by the National Institutes of Health (DK070756) and the US Department of Veterans Affairs.

Disclosures

D.B.M. has been a consultant to ZS Pharma and receives authorship and royalty fees from UpToDate.

References

1. Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O: The rediscovery of uromodulin (Tamm-Horsfall protein): From tubulointerstitial nephropathy to chronic kidney disease. *Kidney Int* 80: 338–347, 2011
2. Nielsen S, Pallone T, Smith BL, Christensen EI, Agre P, Maunsbach AB: Aquaporin-1 water channels in short and long loop descending thin limbs and in descending vasa recta in rat kidney. *Am J Physiol* 268: F1023–F1037, 1995
3. Tsuruoka S, Koseki C, Muto S, Tabei K, Imai M: Axial heterogeneity of potassium transport across hamster thick ascending limb of Henle's loop. *Am J Physiol* 267: F121–F129, 1994
4. Nielsen S, Maunsbach AB, Ecelbarger CA, Knepper MA: Ultrastructural localization of Na-K-2Cl cotransporter in thick ascending limb and macula densa of rat kidney. *Am J Physiol* 275: F885–F893, 1998
5. Yoshitomi K, Kondo Y, Imai M: Evidence for conductive Cl^- pathways across the cell membranes of the thin ascending limb of Henle's loop. *J Clin Invest* 82: 866–871, 1988
6. Jung JY, Song JH, Li C, Yang CW, Kang TC, Won MH, Jeong YG, Han KH, Choi KB, Lee SH, Kim J: Expression of epidermal growth factor in the developing rat kidney. *Am J Physiol Renal Physiol* 288: F227–F235, 2005
7. Ares GR, Caceres PS, Ortiz PA: Molecular regulation of NKCC2 in the thick ascending limb. *Am J Physiol Renal Physiol* 301: F1143–F1159, 2011
8. Burg M, Stoner L, Cardinal J, Green N: Furosemide effect on isolated perfused tubules. *Am J Physiol* 225: 119–124, 1973
9. Hebert SC, Mount DB, Gamba G: Molecular physiology of cation-coupled Cl^- cotransport: The SLC12 family. *Pflügers Arch* 447: 580–593, 2004
10. Plata C, Mount DB, Rubio V, Hebert SC, Gamba G: Isoforms of the Na-K-2Cl cotransporter in murine TAL II. Functional characterization and activation by cAMP. *Am J Physiol* 276: F359–F366, 1999
11. Giménez I, Isenring P, Forbush B: Spatially distributed alternative splice variants of the renal Na-K-Cl cotransporter exhibit dramatically different affinities for the transported ions. *J Biol Chem* 277: 8767–8770, 2002
12. Lapointe JY, Laamarti A, Bell PD: Ionic transport in macula densa cells. *Kidney Int Suppl* 67: S58–S64, 1998
13. Ito S, Carretero OA: An in vitro approach to the study of macula densa-mediated glomerular hemodynamics. *Kidney Int* 38: 1206–1210, 1990
14. He XR, Greenberg SG, Briggs JP, Schnermann J: Effects of furosemide and verapamil on the NaCl dependency of macula densa-mediated renin secretion. *Hypertension* 26: 137–142, 1995
15. Wilcox CS: New insights into diuretic use in patients with chronic renal disease. *J Am Soc Nephrol* 13: 798–805, 2002
16. Okusa MD, Persson AE, Wright FS: Chlorothiazide effect on feedback-mediated control of glomerular filtration rate. *Am J Physiol* 257: F137–F144, 1989
17. Igarashi P, Vanden Heuvel GB, Payne JA, Forbush B 3rd: Cloning, embryonic expression, and alternative splicing of a murine kidney-specific Na-K-Cl cotransporter. *Am J Physiol* 269: F405–F418, 1995
18. Burg MB, Green N: Function of the thick ascending limb of Henle's loop. *Am J Physiol* 224: 659–668, 1973
19. Greger R: Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol Rev* 65: 760–797, 1985
20. Hebert SC, Andreoli TE: Control of NaCl transport in the thick ascending limb. *Am J Physiol* 246: F745–F756, 1984

21. Greger R, Schlatter E: Presence of luminal K⁺, a prerequisite for active NaCl transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pflügers Arch* 392: 92–94, 1981
22. Stokes JB: Consequences of potassium recycling in the renal medulla. Effects of ion transport by the medullary thick ascending limb of Henle's loop. *J Clin Invest* 70: 219–229, 1982
23. Taniguchi J, Guggino WB: Membrane stretch: A physiological stimulator of Ca²⁺-activated K⁺ channels in thick ascending limb. *Am J Physiol* 257: F347–F352, 1989
24. Bleich M, Schlatter E, Greger R: The luminal K⁺ channel of the thick ascending limb of Henle's loop. *Pflügers Arch* 415: 449–460, 1990
25. Wang WH: Two types of K⁺ channel in thick ascending limb of rat kidney. *Am J Physiol* 267: F599–F605, 1994
26. Wang W, Lu M: Effect of arachidonic acid on activity of the apical K⁺ channel in the thick ascending limb of the rat kidney. *J Gen Physiol* 106: 727–743, 1995
27. Ho K, Nichols CG, Lederer WJ, Lytton J, Vassilev PM, Kanazirska MV, Hebert SC: Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature* 362: 31–38, 1993
28. Xu JZ, Hall AE, Peterson LN, Bienkowski MJ, Eessalu TE, Hebert SC: Localization of the ROMK protein on apical membranes of rat kidney nephron segments. *Am J Physiol* 273: F739–F748, 1997
29. Lu M, Wang T, Yan Q, Yang X, Dong K, Knepper MA, Wang W, Giebisch G, Shull GE, Hebert SC: Absence of small conductance K⁺ channel (SK) activity in apical membranes of thick ascending limb and cortical collecting duct in ROMK (Bartter's) knockout mice. *J Biol Chem* 277: 37881–37887, 2002
30. Simon DB, Karet FE, Rodriguez-Soriano J, Hamdan JH, DiPietro A, Trachtman H, Sanjad SA, Lifton RP: Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K⁺ channel, ROMK. *Nat Genet* 14: 152–156, 1996
31. Lu M, Wang T, Yan Q, Wang W, Giebisch G, Hebert SC: ROMK is required for expression of the 70-pS K channel in the thick ascending limb. *Am J Physiol Renal Physiol* 286: F490–F495, 2004
32. Yoo D, Flagg TP, Olsen O, Raghuram V, Foskett JK, Welling PA: Assembly and trafficking of a multiprotein ROMK (Kir 1.1) channel complex by PDZ interactions. *J Biol Chem* 279: 6863–6873, 2004
33. Lu M, Leng Q, Egan ME, Caplan MJ, Boulpaep EL, Giebisch GH, Hebert SC: CFTR is required for PKA-regulated ATP sensitivity of Kir1.1 potassium channels in mouse kidney. *J Clin Invest* 116: 797–807, 2006
34. Nahida R, Mohammed H, Guy L: Pseudo-Bartter's syndrome revealing cystic fibrosis in an infant caused by 3849 + 1G>A and 4382delA compound heterozygosity. *Acta Paediatr* 100: e234–e235, 2011
35. Hebert SC, Culpepper RM, Andreoli TE: NaCl transport in mouse medullary thick ascending limbs. II. ADH enhancement of transcellular NaCl cotransport; origin of transepithelial voltage. *Am J Physiol* 241: F432–F442, 1981
36. Greger R, Schlatter E: Properties of the basolateral membrane of the cortical thick ascending limb of Henle's loop of rabbit kidney. A model for secondary active chloride transport. *Pflügers Arch* 396: 325–334, 1983
37. Greger R, Oberleithner H, Schlatter E, Cassola AC, Weidtkc C: Chloride activity in cells of isolated perfused cortical thick ascending limbs of rabbit kidney. *Pflügers Arch* 399: 29–34, 1983
38. Waldegger S, Jeck N, Barth P, Peters M, Vitzthum H, Wolf K, Kurtz A, Konrad M, Seyberth HW: Barttin increases surface expression and changes current properties of ClC-K channels. *Pflügers Arch* 444: 411–418, 2002
39. Liu W, Morimoto T, Kondo Y, Iinuma K, Uchida S, Sasaki S, Marumo F, Imai M: Analysis of NaCl transport in thin ascending limb of Henle's loop in CLC-K1 null mice. *Am J Physiol Renal Physiol* 282: F451–F457, 2002
40. Kobayashi K, Uchida S, Mizutani S, Sasaki S, Marumo F: Intrarenal and cellular localization of CLC-K2 protein in the mouse kidney. *J Am Soc Nephrol* 12: 1327–1334, 2001
41. Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonca E, Stone R, Schurman S, Nayir A, Alpay H, Bakkaloglu A, Rodriguez-Soriano J, Morales JM, Sanjad SA, Taylor CM, Pilz D, Brem A, Trachtman H, Griswold W, Richard GA, John E, Lifton RP: Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. *Nat Genet* 17: 171–178, 1997
42. Palmer LG, Frindt G: Cl[−] channels of the distal nephron. *Am J Physiol Renal Physiol* 291: F1157–F1168, 2006
43. Estévez R, Boettger T, Stein V, Birkenhäger R, Otto E, Hildebrandt F, Jentsch TJ: Barttin is a Cl[−] channel beta-subunit crucial for renal Cl[−] reabsorption and inner ear K⁺ secretion. *Nature* 414: 558–561, 2001
44. Picollo A, Liantonio A, Didonna MP, Elia L, Camerino DC, Pusch M: Molecular determinants of differential pore blocking of kidney CLC-K chloride channels. *EMBO Rep* 5: 584–589, 2004
45. Di Stefano A, Greger R, Desfleurs E, de Rouffignac C, Wittner M: A Ba(2+)-insensitive K⁺ conductance in the basolateral membrane of rabbit cortical thick ascending limb cells. *Cell Physiol Biochem* 8: 89–105, 1998
46. Mercado A, Song L, Vazquez N, Mount DB, Gamba G: Functional comparison of the K⁺-Cl[−] cotransporters KCC1 and KCC4. *J Biol Chem* 275: 30326–30334, 2000
47. Guggino WB: Functional heterogeneity in the early distal tubule of the Amphiuma kidney: Evidence for two modes of Cl[−] and K⁺ transport across the basolateral cell membrane. *Am J Physiol* 250: F430–F440, 1986
48. Cabral PD, Herrera M: Membrane-associated aquaporin-1 facilitates osmotically driven water flux across the basolateral membrane of the thick ascending limb. *Am J Physiol Renal Physiol* 303: F621–F629, 2012
49. Sun A, Grossman EB, Lombardi M, Hebert SC: Vasopressin alters the mechanism of apical Cl[−] entry from Na⁺:Cl[−] to Na⁺:K⁺:2Cl[−] cotransport in mouse medullary thick ascending limb. *J Membr Biol* 120: 83–94, 1991
50. Hebert SC, Andreoli TE: Ionic conductance pathways in the mouse medullary thick ascending limb of Henle. The paracellular pathway and electrogenic Cl[−] absorption. *J Gen Physiol* 87: 567–590, 1986
51. Furuse M, Furuse K, Sasaki H, Tsukita S: Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. *J Cell Biol* 153: 263–272, 2001
52. Konrad M, Schaller A, Seelow D, Pandey AV, Waldegger S, Lesslauer A, Vitzthum H, Suzuki Y, Luk JM, Becker C, Schlingmann KP, Schmid M, Rodriguez-Soriano J, Ariceta G, Cano F, Enriquez R, Juppner H, Bakkaloglu SA, Hediger MA, Gallati S, Neuhauss SC, Nurnberg P, Weber S: Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet* 79: 949–957, 2006
53. Angelow S, El-Husseini R, Kanzawa SA, Yu AS: Renal localization and function of the tight junction protein, claudin-19. *Am J Physiol Renal Physiol* 293: F166–F177, 2007
54. Breiderhoff T, Himmerkus N, Stuiver M, Mutig K, Will C, Meij IC, Bachmann S, Bleich M, Willnow TE, Müller D: Deletion of claudin-10 (Cldn10) in the thick ascending limb impairs paracellular sodium permeability and leads to hypermagnesemia and nephrocalcinosis. *Proc Natl Acad Sci U S A* 109: 14241–14246, 2012
55. Hou J, Paul DL, Goodenough DA: Paracellin-1 and the modulation of ion selectivity of tight junctions. *J Cell Sci* 118: 5109–5118, 2005
56. Hou J, Renigunta A, Konrad M, Gomes AS, Schneeberger EE, Paul DL, Waldegger S, Goodenough DA: Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. *J Clin Invest* 118: 619–628, 2008
57. Hou J, Renigunta A, Gomes AS, Hou M, Paul DL, Waldegger S, Goodenough DA: Claudin-16 and claudin-19 interaction is required for their assembly into tight junctions and for renal reabsorption of magnesium. *Proc Natl Acad Sci U S A* 106: 15350–15355, 2009
58. Himmerkus N, Shan Q, Goerke B, Hou J, Goodenough DA, Bleich M: Salt and acid-base metabolism in claudin-16 knockdown mice: Impact for the pathophysiology of FHHNC patients. *Am J Physiol Renal Physiol* 295: F1641–F1647, 2008

59. Gong Y, Renigunta V, Himmerkus N, Zhang J, Renigunta A, Bleich M, Hou J: Claudin-14 regulates renal Ca^{2+} transport in response to CaSR signalling via a novel microRNA pathway. *EMBO J* 31: 1999–2012, 2012
60. Toka HR, Al-Romaih K, Koshy JM, DiBartolo S 3rd, Kos CH, Quinn SJ, Curhan GC, Mount DB, Brown EM, Pollak MR: Deficiency of the calcium-sensing receptor in the kidney causes parathyroid hormone-independent hypocalciuria. *J Am Soc Nephrol* 23: 1879–1890, 2012
61. Dimke H, Desai P, Borovac J, Lau A, Pan W, Alexander RT: Activation of the Ca^{2+} -sensing receptor increases renal claudin-14 expression and urinary Ca^{2+} excretion. *Am J Physiol Renal Physiol* 304: F761–F769, 2013
62. Good DW: Sodium-dependent bicarbonate absorption by cortical thick ascending limb of rat kidney. *Am J Physiol* 248: F821–F829, 1985
63. Wang T, Hropot M, Aronson PS, Giebisch G: Role of NHE isoforms in mediating bicarbonate reabsorption along the nephron. *Am J Physiol Renal Physiol* 281: F1117–F1122, 2001
64. Bourgeois S, Massé S, Paillard M, Houillier P: Basolateral membrane Cl^{-} -, Na^{+} -, and K^{+} -coupled base transport mechanisms in rat MTALH. *Am J Physiol Renal Physiol* 282: F655–F668, 2002
65. Odgaard E, Jakobsen JK, Frische S, Praetorius J, Nielsen S, Aalkjaer C, Leipziger J: Basolateral Na^{+} -dependent HCO_3^{-} -transporter NBCn1-mediated HCO_3^{-} -influx in rat medullary thick ascending limb. *J Physiol* 555: 205–218, 2004
66. Capasso G, Unwin R, Ciani F, De Santo NG, De Tommaso G, Russo F, Giebisch G: Bicarbonate transport along the loop of Henle. II. Effects of acid-base, dietary, and neurohumoral determinants. *J Clin Invest* 94: 830–838, 1994
67. Good DW: Ammonium transport by the thick ascending limb of Henle's loop. *Annu Rev Physiol* 56: 623–647, 1994
68. Packer RK, Desai SS, Hornbuckle K, Knepper MA: Role of countercurrent multiplication in renal ammonium handling: Regulation of medullary ammonium accumulation. *J Am Soc Nephrol* 2: 77–83, 1991
69. Biver S, Belge H, Bourgeois S, Van Vooren P, Nowik M, Scohy S, Houillier P, Szpirer J, Szpirer C, Wagner CA, Devuyst O, Marini AM: A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility. *Nature* 456: 339–343, 2008
70. Attmane-Elakeb A, Mount DB, Sibella V, Vernimmen C, Hebert SC, Bichara M: Stimulation by in vivo and in vitro metabolic acidosis of expression of rBSC-1, the Na^{+} - K^{+} -(NH_4^{+})- 2Cl^{-} -cotransporter of the rat medullary thick ascending limb. *J Biol Chem* 273: 33681–33691, 1998
71. Bourgeois S, Meer LV, Wootla B, Bloch-Faure M, Chambrey R, Shull GE, Gawenis LR, Houillier P: NHE4 is critical for the renal handling of ammonia in rodents. *J Clin Invest* 120: 1895–1904, 2010
72. Bettinelli A, Ciarmatori S, Cesareo L, Tedeschi S, Ruffa G, Appiani AC, Rosini A, Grumieri G, Mercuri B, Sacco M, Leozappa G, Binda S, Cecconi M, Navone C, Curcio C, Syren ML, Casari G: Phenotypic variability in Bartter syndrome type I. *Pediatr Nephrol* 14: 940–945, 2000
73. DuBose TD Jr, Good DW: Chronic hyperkalemia impairs ammonium transport and accumulation in the inner medulla of the rat. *J Clin Invest* 90: 1443–1449, 1992
74. Szyman P, Better OS, Chaimowitz C, Rosler A: Role of hyperkalemia in the metabolic acidosis of isolated hypoaldosteronism. *N Engl J Med* 294: 361–365, 1976
75. Buysse JM, Huang IZ, Pitt B: PEARL-HF: Prevention of hyperkalemia in patients with heart failure using a novel polymeric potassium binder, RLY5016. *Future Cardiol* 8: 17–28, 2012
76. Féraille E, Doucet A: Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: Hormonal control. *Physiol Rev* 81: 345–418, 2001
77. Silva GB, Garvin JL: Angiotensin II-dependent hypertension increases Na^{+} transport-related oxygen consumption by the thick ascending limb. *Hypertension* 52: 1091–1098, 2008
78. Wu P, Wang M, Luan H, Li L, Wang L, Wang WH, Gu R: Angiotensin II stimulates basolateral 10-pS Cl^{-} channels in the thick ascending limb. *Hypertension* 61: 1211–1217, 2013
79. Mutig K, Paliege A, Kahl T, Jöns T, Müller-Esterl W, Bachmann S: Vasopressin V2 receptor expression along rat, mouse, and human renal epithelia with focus on TAL. *Am J Physiol Renal Physiol* 293: F1166–F1177, 2007
80. Giménez I, Forbush B: Short-term stimulation of the renal Na^{+} - K^{+} - Cl^{-} cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. *J Biol Chem* 278: 26946–26951, 2003
81. Gagnon KB, England R, Delpire E: Volume sensitivity of cation- Cl^{-} cotransporters is modulated by the interaction of two kinases: Ste20-related proline-alanine-rich kinase and WNK4. *Am J Physiol Cell Physiol* 290: C134–C142, 2006
82. Delpire E, Gagnon KB: Genome-wide analysis of SPAK/OSR1 binding motifs. *Physiol Genomics* 28: 223–231, 2007
83. Gagnon KB, England R, Delpire E: A single binding motif is required for SPAK activation of the Na^{+} - K^{+} - 2Cl^{-} cotransporter. *Cell Physiol Biochem* 20: 131–142, 2007
84. Reiche J, Theilig F, Rafiqi FH, Carlo AS, Militz D, Mutig K, Todiras M, Christensen EI, Ellison DH, Bader M, Nykjaer A, Bachmann S, Alessi D, Willnow TE: SORLA/SORL1 functionally interacts with SPAK to control renal activation of Na^{+} - K^{+} - Cl^{-} cotransporter 2. *Mol Cell Biol* 30: 3027–3037, 2010
85. Lin SH, Yu IS, Jiang ST, Lin SW, Chu P, Chen A, Sytwu HK, Sohara E, Uchida S, Sasaki S, Yang SS: Impaired phosphorylation of Na^{+} - K^{+} - 2Cl^{-} cotransporter by oxidative stress-responsive kinase-1 deficiency manifests hypotension and Bartter-like syndrome. *Proc Natl Acad Sci U S A* 108: 17538–17543, 2011
86. Rafiqi FH, Zuber AM, Glover M, Richardson C, Fleming S, Jovanović S, Jovanović A, O'Shaughnessy KM, Alessi DR: Role of the WNK-activated SPAK kinase in regulating blood pressure. *EMBO Mol Med* 2: 63–75, 2010
87. Ponce-Coria J, San-Cristobal P, Kahle KT, Vazquez N, Pacheco-Alvarez D, de Los Heros P, Juárez P, Muñoz E, Michel G, Bobadilla NA, Gimenez I, Lifton RP, Hebert SC, Gamba G: Regulation of NKCC2 by a chloride-sensing mechanism involving the WNK3 and SPAK kinases. *Proc Natl Acad Sci U S A* 105: 8458–8463, 2008
88. Ponce-Coria J, Gagnon KB, Delpire E: Calcium-binding protein 39 facilitates molecular interaction between Ste20p proline alanine-rich kinase and oxidative stress response 1 monomers. *Am J Physiol Cell Physiol* 303: C1198–C1205, 2012
89. Gunaratne R, Braucht DW, Rinschen MM, Chou CL, Hoffer JD, Pisitkun T, Knepper MA: Quantitative phosphoproteomic analysis reveals cAMP/vasopressin-dependent signaling pathways in native renal thick ascending limb cells. *Proc Natl Acad Sci U S A* 107: 15653–15658, 2010
90. Knepper MA, Kim GH, Fernández-Llana P, Ecelbarger CA: Regulation of thick ascending limb transport by vasopressin. *J Am Soc Nephrol* 10: 628–634, 1999
91. Hebert SC: Calcium and salinity sensing by the thick ascending limb: A journey from mammals to fish and back again. *Kidney Int Suppl* 91 (Suppl): S28–S33, 2004
92. Loupy A, Ramakrishnan SK, Wootla B, Chambrey R, de la Faille R, Bourgeois S, Bruneval P, Mandet C, Christensen EI, Faure H, Cheval L, Laghmani K, Collet C, Eladari D, Dodd RH, Ruat M, Houillier P: PTH-independent regulation of blood calcium concentration by the calcium-sensing receptor. *J Clin Invest* 122: 3355–3367, 2012
93. de Jesus Ferreira MC, Héliers-Toussaint C, Imbert-Teboul M, Bailly C, Verbavatz JM, Bellanger AC, Chabardès D: Co-expression of a Ca^{2+} -inhibitable adenylyl cyclase and of a Ca^{2+} -sensing receptor in the cortical thick ascending limb cell of the rat kidney. Inhibition of hormone-dependent cAMP accumulation by extracellular Ca^{2+} . *J Biol Chem* 273: 15192–15202, 1998
94. Gu RM, Yang L, Zhang Y, Wang L, Kong S, Zhang C, Zhai Y, Wang M, Wu P, Liu L, Gu F, Zhang J, Wang WH: CYP-omega-hydroxylation-dependent metabolites of arachidonic acid inhibit the basolateral 10 pS Cl^{-} channel in the rat thick ascending limb. *Kidney Int* 76: 849–856, 2009
95. Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, Okazaki R, Chikatsu N, Fujita T: Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet* 360: 692–694, 2002
96. Vargas-Poussou R, Huang C, Hulin P, Houillier P, Jeunemaître X, Paillard M, Planelles G, Déchaux M, Miller RT, Antignac C: Functional characterization of a calcium-sensing receptor

- mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol* 13: 2259–2266, 2002
97. Thorleifsson G, Holm H, Edvardsson V, Walters GB, Styrkarsdottir U, Gudbjartsson DF, Sulem P, Halldorsson BV, de Vegt F, d'Ancona FC, den Heijer M, Franzson L, Christiansen C, Alexandersen P, Rafnar T, Kristjansson K, Sigurdsson G, Kiemenev LA, Bodvarsson M, Indridason OS, Palsson R, Kong A, Thorsteinsdottir U, Stefansson K: Sequence variants in the CLDN14 gene associate with kidney stones and bone mineral density. *Nat Genet* 41: 926–930, 2009
 98. Mutig K, Kahl T, Saritas T, Godes M, Persson P, Bates J, Raffi H, Rampoldi L, Uchida S, Hille C, Dosche C, Kumar S, Castañeda-Bueno M, Gamba G, Bachmann S: Activation of the bumetanide-sensitive Na⁺,K⁺,2Cl⁻ cotransporter (NKCC2) is facilitated by Tamm-Horsfall protein in a chloride-sensitive manner. *J Biol Chem* 286: 30200–30210, 2011
 99. Renigunta A, Renigunta V, Saritas T, Decher N, Mutig K, Waldegger S: Tamm-Horsfall glycoprotein interacts with renal outer medullary potassium channel ROMK2 and regulates its function. *J Biol Chem* 286: 2224–2235, 2011
 100. Wolf MT, Wu XR, Huang CL: Uromodulin upregulates TRPV5 by impairing caveolin-mediated endocytosis. *Kidney Int* 84: 130–137, 2013
 101. Ying WZ, Allen CE, Curtis LM, Aaron KJ, Sanders PW: Mechanism and prevention of acute kidney injury from cast nephropathy in a rodent model. *J Clin Invest* 122: 1777–1785, 2012
 102. Dahan K, Devuyst O, Smaers M, Vertommen D, Loute G, Poux JM, Viron B, Jacquot C, Gagnadoux MF, Chauveau D, Büchler M, Cochat P, Cosyns JP, Mougenot B, Rider MH, Antignac C, Verellen-Dumoulin C, Pirson Y: A cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J Am Soc Nephrol* 14: 2883–2893, 2003
 103. Trudu M, Janas S, Lanzani C, Debaix H, Schaeffer C, Ikehata M, Citterio L, Demaretz S, Trevisani F, Ristagno G, Glaudemans B, Laghmani K, Dell'Antonio G, Loffing J, Rastaldi MP, Manunta P, Devuyst O, Rampoldi L; Swiss Kidney Project on Genes in Hypertension (SKIPOGH) team: Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. *Nat Med* 19: 1655–1660, 2013
 104. Olden M, Corre T, Hayward C, Toniolo D, Ulivi S, Gasparini P, Pistis G, Hwang SJ, Bergmann S, Campbell H, Cocca M, Gandin I, Girotto G, Glaudemans B, Hastie ND, Loffing J, Polasek O, Rampoldi L, Rudan I, Sala C, Traglia M, Vollenweider P, Vuckovic D, Youhanna S, Weber J, Wright AF, Kutalik Z, Bochud M, Fox CS, Devuyst O: Common variants in UMOD associate with urinary uromodulin levels: A meta-analysis. *J Am Soc Nephrol* 25: 1869–1882, 2014
 105. Suki WN, Yium JJ, Von Minden M, Saller-Hebert C, Eknayan G, Martinez-Maldonado M: Acute treatment of hypercalcemia with furosemide. *N Engl J Med* 283: 836–840, 1970
 106. Decaux G, Waterlot Y, Genette F, Hallemans R, Demanet JC: Inappropriate secretion of antidiuretic hormone treated with frusemide. *Br Med J (Clin Res Ed)* 285: 89–90, 1982
 107. Hwang SJ, Haas M, Harris HW Jr, Silva P, Yalla S, Sullivan MR, Otuechere G, Kashgarian M, Zeidel ML: Transport defects of rabbit medullary thick ascending limb cells in obstructive nephropathy. *J Clin Invest* 91: 21–28, 1993
 108. Guay-Woodford LM: Bartter syndrome: Unraveling the pathophysiological enigma. *Am J Med* 105: 151–161, 1998
 109. Meyer WJ 3rd, Gill JR Jr, Bartter FC: Gout as a complication of Bartter's syndrome. A possible role for alkalosis in the decreased clearance of uric acid. *Ann Intern Med* 83: 56–59, 1975
 110. Tasic V, Dervisov D, Koceva S, Weber S, Konrad M: Hypomagnesemia with hypercalciuria and nephrocalcinosis: Case report and a family study. *Pediatr Nephrol* 20: 1003–1006, 2005

Published online ahead of print. Publication date available at www.cjasn.org.



Distal Convoluted Tubule

Arohan R. Subramanya^{*†‡} and David H. Ellison^{§||¶}

Abstract

The distal convoluted tubule is the nephron segment that lies immediately downstream of the macula densa. Although short in length, the distal convoluted tubule plays a critical role in sodium, potassium, and divalent cation homeostasis. Recent genetic and physiologic studies have greatly expanded our understanding of how the distal convoluted tubule regulates these processes at the molecular level. This article provides an update on the distal convoluted tubule, highlighting concepts and pathophysiology relevant to clinical practice.

Clin J Am Soc Nephrol 9: 2147–2163, 2014. doi: 10.2215/CJN.05920613

Introduction

The distal convoluted tubule (DCT) is the portion of the nephron that is immediately downstream of the macula densa. Although the DCT is the shortest segment of the nephron, spanning only about 5 mm in length in humans (1), it plays a critical role in a variety of homeostatic processes, including sodium chloride reabsorption, potassium secretion, and calcium and magnesium handling. The DCT has a unique capacity to adapt to changes in hormonal stimuli and the contents of the tubular lumen, and this process contributes to the pathophysiology of a number of clinically relevant scenarios, including loop diuretic resistance and hyperaldosteronism. In recent years, insights gained from Mendelian disorders of BP and electrolyte imbalance, genetically modified animal models, and molecular cloning and study of key components of the machinery that controls the diverse ion transport processes housed in this segment have greatly expanded our understanding of distal tubule function. In this article, we provide a focused overview and update of the molecular physiology of the DCT.

Distal Nephron Nomenclature and Anatomic Considerations

The term distal tubule has been used by anatomists to denote the region of the nephron that extends downstream from the macula densa to the confluence of another tubule (*i.e.*, the collecting system) (Figure 1) (2). It includes two nephron segments, the DCT and the connecting tubule (CNT). The DCT can be further divided into two functionally distinct subsegments, referred to as the DCT1 and the DCT2 (also called the early and late DCTs).

The DCT1 and DCT2 can be distinguished by their differential responsiveness to the mineralocorticoid aldosterone. Aldosterone is a steroid hormone that is released from the adrenal gland in response to volume depletion or hyperkalemia. It has a similar chemical

structure to glucocorticoids, such as cortisol, and both aldosterone and cortisol bind to the mineralocorticoid receptor with nearly equal affinity (3). Although mineralocorticoid receptors are expressed throughout the entire DCT, the DCT2 is sensitive to the actions of aldosterone, because it expresses an enzyme called 11- β hydroxysteroid dehydrogenase 2 (11- β HSD2). 11- β HSD2 metabolizes cortisol to the inactive metabolite cortisone, thereby preventing circulating glucocorticoids from binding to mineralocorticoid receptors expressed in the DCT2 (4). Because the mineralocorticoid receptors in the DCT2 can only be occupied by aldosterone, the DCT2 is more sensitive than the DCT1 to changes in circulating aldosterone levels. 11- β HSD2 is also expressed in other downstream nephron segments, including the CNT and the cortical collecting duct (CCD). For this reason, the DCT2, CNT, and CCD are collectively termed the aldosterone-sensitive distal nephron (5).

Cells of the DCT have a unique morphology that matches their highly active physiology (Figure 1, insets) (6). Their nuclei tend to be positioned to the apical side of the cell because of an extensive basolateral membrane that has numerous deep infoldings. In addition, the cytosol on the basal aspect of DCT cells is packed with mitochondria—in fact, cells of the DCT are among the most mitochondria-rich in the kidney (7). These findings indicate that the DCT engages in processes that require considerable ATP consumption and active transport of electrolytes driven by the basolateral Na⁺-K⁺-ATPase.

Mechanism of Na⁺ Reabsorption in the DCT Apical Sodium Transport in the Early and Late DCTs

The DCT reabsorbs roughly 5%–10% of the filtered sodium load (8). The thiazide-sensitive NaCl cotransporter (NCC; TSC; SLC12A3) is chiefly responsible for this process. NCC is one of the major targets of thiazide-type diuretics; the other target is sodium-dependent chloride bicarbonate exchanger (NDCBE; SLC4A8), a

Departments of
*Medicine and [†]Cell
Biology, University of
Pittsburgh School of
Medicine, Pittsburgh,
Pennsylvania;
[‡]Veterans Affairs
Pittsburgh Healthcare
System, Pittsburgh,
Pennsylvania;
Departments of
[§]Medicine and
^{||}Physiology and
Pharmacology,
Oregon Health and
Science University,
Portland, Oregon; and
[¶]Portland Veterans
Affairs Medical
Center, Portland,
Oregon

Correspondence:
Dr. Arohan R.
Subramanya,
Department of
Medicine, Renal-
Electrolyte Division,
University of
Pittsburgh School of
Medicine, S828A
Scaife Hall, 3550
Terrace Street,
Pittsburgh, PA 15261.
Email: arsl29@pitt.
edu

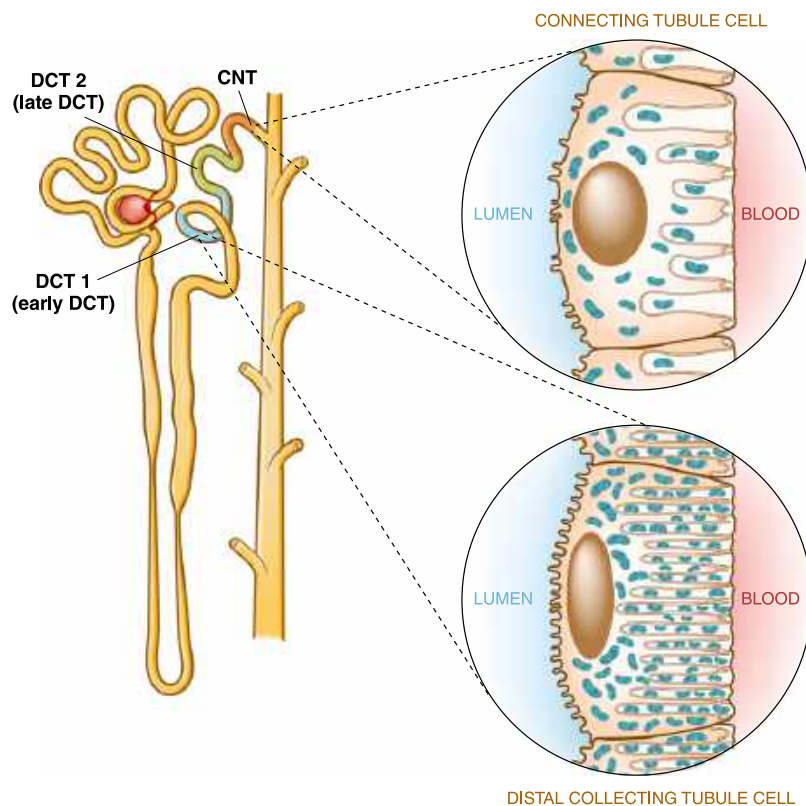


Figure 1. | A human nephron, showing the anatomic location of the distal tubule. The distal convoluted tubule (DCT) is divided into early and late segments, termed DCT1 and DCT2, respectively. The connecting tubule (CNT) is located immediately downstream of the DCT2. Insets compare DCT and CNT cell morphology. The cells of the DCT contain an extensive basolateral membrane system and are mitochondria-rich, indicating high transport activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$. CNT cells, in contrast, contain fewer mitochondria and basolateral membrane invaginations, suggesting that they are less metabolically active.

recently discovered Na^+ -driven chloride bicarbonate exchanger expressed in the CCD (9). This class of drugs is commonly used to treat hypertension, edema, and nephrolithiasis and include the thiazides chlorothiazide, hydrochlorothiazide, and bendroflumethiazide (only available combined with nadolol in the United States) as well as the thiazide-like diuretics metolazone, indapamide, and chlorthalidone. As shown in Figure 2, in the DCT1, sodium absorption from the tubular lumen is chloride-dependent and entirely mediated by NCC (10). Because a negatively charged chloride anion enters in 1:1 stoichiometry with a sodium cation, this process is electrically silent or *electroneutral*. Loss-of-function mutations of NCC cause Gitelman syndrome, an autosomal recessive salt wasting disorder that commonly presents with low-to-normal BP, elevated renin levels, hypokalemia, metabolic alkalosis, hypocalciuria, and hypomagnesemia (11). Thus, the phenotype of Gitelman syndrome is similar to the effects of thiazide diuretics. Most Gitelman mutations introduce changes into the NCC polypeptide that cause the cotransporter to misfold (12). This results in enhanced recognition by a molecular chaperone protein called Hsp70, which binds to misfolded NCC as it is being made in the endoplasmic reticulum and targets it for degradation (13,14).

NCC is expressed throughout the DCT, but the DCT2 also exhibits an amiloride-sensitive sodium transport pathway mediated by the epithelial sodium channel (ENaC)

(Figure 2) (15,16). ENaC-mediated sodium transport is *electrogenic*; thus, in the DCT2, movement of Na^+ ions through ENaC without an accompanying anion leaves negative charges in the lumen. Therefore, the transepithelial voltage or sum of membrane potentials on the luminal and basolateral membranes of the DCT epithelium is lumen-negative. As will be discussed below, this electrical gradient provides a driving force for the movement of other ions. Because ENaC is also expressed in the CNT and collecting duct, the transepithelial voltage becomes progressively more lumen-negative downstream of the DCT (17,18).

Basolateral Sodium Transport

In both subsegments of the DCT, after the transapical movement of Na^+ through NCC and/or ENaC, Na^+ is extruded from the cytosol and into the peritubular fluid by the $\text{Na}^+\text{-K}^+\text{-ATPase}$ (Figure 2). Because this electrogenic pump exchanges sodium for potassium at a stoichiometry of 3:2, its activity removes one positive charge from the intracellular space, resulting in the generation of a constant negative voltage of -60 to -90 mV across the basolateral membrane (19,20). Along the basolateral membrane, the intracellular voltage becomes more negative with respect to extracellular voltage if the intracellular sodium concentration increases. This finding suggests that, in the DCT, a rise in luminal NCC- or ENaC-mediated sodium transport stimulates basolateral Na^+ extrusion through the $\text{Na}^+\text{-K}^+\text{-ATPase}$ (19). This

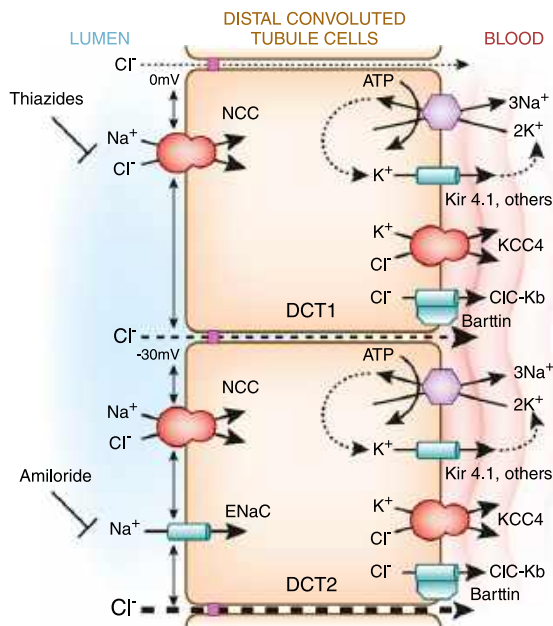


Figure 2. | A model of NaCl reabsorption by cells of the early and late DCTs. Note that the transepithelial voltage is close to zero in the DCT1 and becomes progressively negative in the DCT2. In the early DCT, apical sodium reabsorption is exclusively mediated by thiazide-sensitive NaCl cotransporter (NCC), whereas in the late DCT, both NCC and amiloride-sensitive epithelial sodium channels (ENaCs) are present. The electrogenic transport of sodium by ENaC contributes to the lumen-negative transepithelial voltage. Basolateral sodium efflux is mediated by the $\text{Na}^+\text{-K}^+\text{-ATPase}$ and aided by Kir4.1-mediated potassium leak currents. Chloride transport is carried out by the chloride channel CIC-Kb and potassium chloride cotransporter 4 (KCC4; SLC12A7).

provides an effective mechanism by which sodium ions can be transported transcellularly (*i.e.*, through cells) and returned back to the plasma.

Basolateral K^+ Channels

In order for sodium to be reabsorbed through transcellular mechanisms, basolateral sodium transport through the $\text{Na}^+\text{-K}^+\text{-ATPase}$ must match the rate of apical sodium flux. Basolateral K^+ efflux pathways play an important role in this process (21). By transporting K^+ extracellularly, basolateral potassium channels create a “leak” that recycles K^+ and helps to maintain the activity of the sodium pump (Figure 2). This so-called “pump leak coupling” maximizes the sodium reabsorptive capacity of an epithelium (22). Although renal physiologists have long appreciated this concept (23), its medical relevance only recently came to light through studies of a rare genetic disorder called EAST/SeSAME (24,25). In 2008, two groups reported that patients with mutations in the DCT-expressed basolateral inward-rectifying K^+ channel Kir4.1 (KCNJ10) develop a hereditary salt wasting hypokalemic and hypomagnesemic disorder resembling Gitelman syndrome (24,25). Patients with these mutations typically present in infancy with a constellation of neurologic abnormalities in addition to the renal salt loss; for this reason, the disease has been named EAST syndrome (epilepsy,

ataxia, sensorineural deafness, and tubulopathy) by some investigators and SeSAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) by others. The Gitelman-like phenotype seen in individuals with the syndrome is likely caused by impaired Kir4.1 function and potassium recycling at the basolateral membrane of the DCT (26). In the absence of Kir4.1 activity, the activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ is reduced, resulting in diminished transcellular Na^+ reabsorption in the DCT (Figure 3). Recent studies indicate that patients with EAST/SeSAME syndrome develop markedly reduced infoldings of the basolateral membrane of the DCT (26). This reduction in basolateral membrane surface area leads to a decrease in the total number of surface-localized $\text{Na}^+\text{-K}^+\text{-ATPase}$ molecules, providing a second reason why basolateral sodium transport is reduced in these patients (Figure 3). To date, EAST/SeSAME syndrome is the only clinical distal tubular disorder known to affect a basolateral potassium channel. However, Kir4.1 is not the only potassium channel expressed at the basolateral membrane of the DCT. In fact, a number of K^+ channels are expressed basolaterally, including Kir4.2 (KCNJ15) and Kir5.1 (KCNJ16) (21). Kir4.1 may associate in complexes with these alternative K^+ transport pathways to form heteromultimers that cooperate in basolateral K^+ recycling (27).

Cl^- Absorption in the DCT

Apical Chloride Transport Pathways

In the early DCT, chloride is transported transcellularly from the tubular lumen with sodium through NCC. Sodium and chloride transport through this cotransporter is interdependent; thus, inhibition of NCC by thiazide diuretics will effectively block chloride reabsorption in the earliest loops of the distal nephron. As mentioned above, on reaching the late DCT, sodium is also transported through amiloride-sensitive ENaCs, which generate a lumen-negative transepithelial potential. This, in turn, serves as a driving force for chloride transport into the peritubular space through paracellular mechanisms (Figure 2). Paracellular transport (*i.e.*, the transport of ions between cells) is a passive process, but cells generally confer specificity to the process by expressing multiprotein complexes at or near the tight junctions that connect adjacent cells. Included among these complexes is a large family of molecules called claudins (28). Current data indicate that different regions of the nephron express specific combinations of claudin proteins, which help to define the paracellular ion selectivities of those nephron segments.

Basolateral Chloride Transport

Because sodium transport in the DCT largely occurs through the coupled transcellular transport of sodium and chloride, basolateral Cl^- transport is essential to the development of a gradient for Na^+ entry. Cl^- exit also helps to lower the intracellular Cl^- concentration, which is a potent activator of NCC, and maintains net Na^+ reabsorption (29). Cl^- exit across the human DCT basolateral membrane is mediated by the chloride channel CIC-Kb. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ is necessary for this process, because it generates an electrochemical gradient that favors chloride efflux. CIC-Kb channels require an accessory subunit called Barttin to be fully

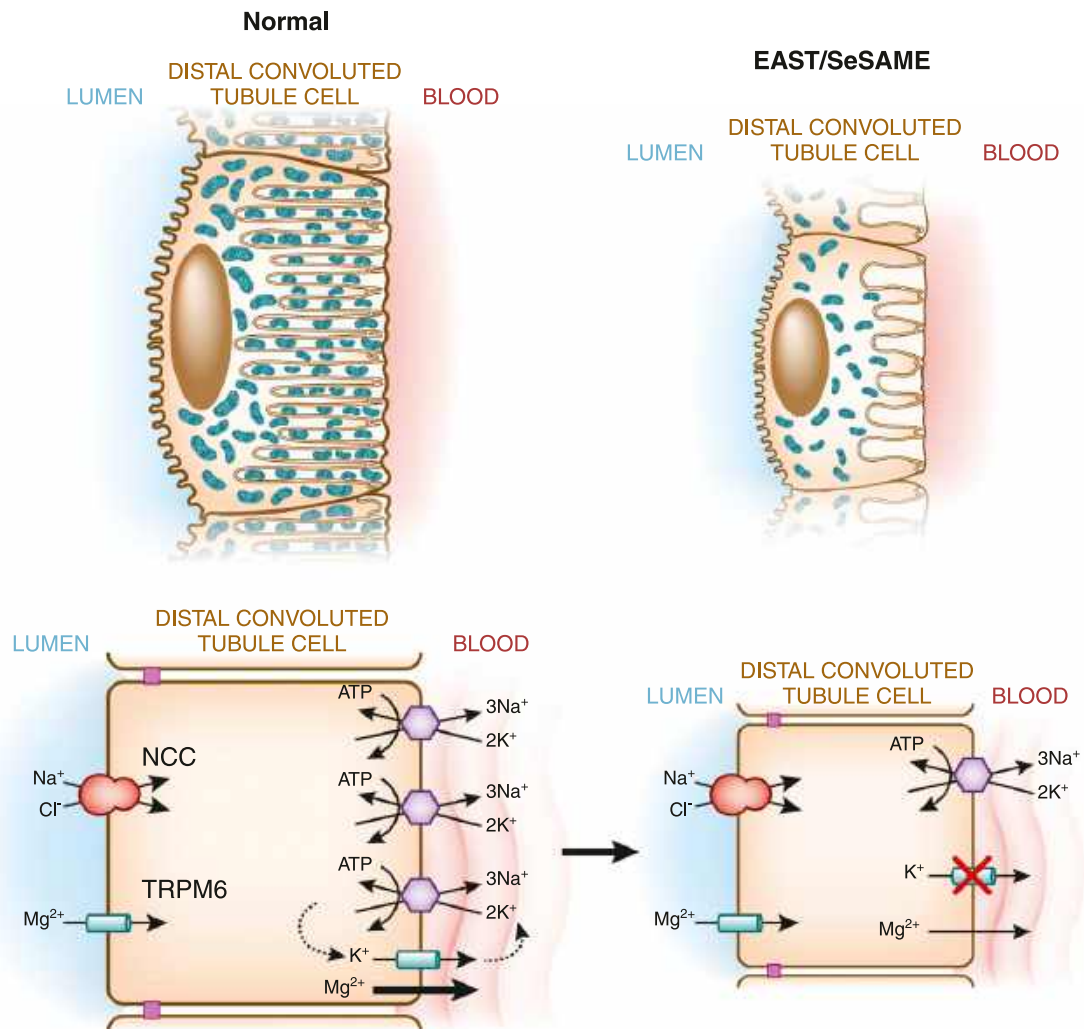


Figure 3. | Mechanism of diminished NaCl and Mg²⁺ reabsorption in EAST/SeSAME syndrome. EAST/SeSAME syndrome is caused by mutations of the basolateral K⁺ channel (KCNJ10; Kir4.1). This channel mediates leakage of potassium to the peritubular fluid, which provides a supply of potassium ions that drives the activity of the Na⁺-K⁺-ATPase, resulting in constant reabsorption of sodium across the basolateral membrane. In EAST/SeSAME syndrome, inactivating mutations of Kir4.1 impair a leak current, which probably reduces activity of the sodium/potassium pump. Patients with the syndrome also display a reduced basolateral membrane surface area, consistent with diminished Na⁺-K⁺-ATPase activity. The reduction in basolateral sodium transport reduces the gradient for NCC-mediated Na⁺ reabsorption and causes salt wasting. Luminal magnesium reabsorption is similarly reduced in these patients, possibly because of diminished basolateral magnesium transport. EAST/SeSAME, epilepsy, ataxia, sensorineural deafness, and tubulopathy/seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance.

functional (30). Both the ClC-Kb channel and Barttin are also expressed in the thick ascending limb. Thus, it is perhaps not surprising that loss-of-function mutations of either gene cause specific subtypes of Bartter syndrome (30,31). Patients with ClC-Kb channel mutations tend to present with a mixed Bartter/Gitelman phenotype, whereas patients lacking functional Barttin present with a severe neonatal salt wasting disorder that includes sensorineural deafness.

The potassium chloride cotransporter 4 (KCC4; SLC12A7) is expressed in the basolateral membrane of the DCT and mediates coupled electroneutral K-Cl efflux (32). KCC4 activity is stimulated by hypotonicity (33). Because luminal fluid in the DCT is hypotonic relative to plasma (34), the extracellular environment of the DCT theoretically favors KCC activation. This finding is particularly relevant in the DCT2, where tubular fluid is diluted down to 100 mOsm.

Thus, a significant fraction of basolateral chloride reabsorption in the late DCT probably occurs through this transport pathway. Although no clinical syndrome involving KCC4 mutations has been described, KCC4 knockout mice develop renal tubular acidosis and deafness (35). This suggests that KCC4 plays an important role in determining renal net acid excretion and K⁺ secretion into the endolymph of the inner ear, although the mechanisms mediating such processes remain poorly defined.

Regulation of NaCl Transport in the DCT

Although sodium transport in the DCT is mediated by both NCC and ENaC, in this section, we will predominantly focus on the regulation of NCC-mediated Na⁺ transport. A detailed review of ENaC regulation is covered elsewhere in this series.

Distal Na⁺ Delivery

NaCl reabsorption in the DCT is dependent on sodium delivery. As the delivered sodium load is increased, the DCT responds by increasing its capacity for sodium transport. These changes are associated with marked alterations in DCT cell morphology, including an increase in basolateral membrane surface area and increased mitochondrial size (36). In concordance with these hypertrophic changes, Na⁺-K⁺-ATPase activity and the number of NCC cotransporters expressed per cell both increase dramatically. Thus, luminal sodium seems to be a potent stimulus that induces hypertrophy and activity of the distal tubule. The cellular mechanisms whereby NaCl entry affects NCC transport function and DCT morphology are not known.

The most common clinical scenario in which this phenomenon is encountered is loop diuretic resistance (Figure 4) (37). Chronic treatment with diuretics that inhibit the Na-K-2Cl cotransporter in the loop of Henle (bumetanide-sensitive sodium-potassium-chloride cotransporter [NKCC2, SLC12A1]), such as furosemide, results in a sustained increase in luminal Na⁺ delivery to the DCT. This induces hypertrophic changes in the DCT that increase NCC-mediated NaCl reabsorption, diminishing the effects of loop diuretics over time (36). Because of these hypertrophic changes, edematous patients who are loop diuretic-resistant and do not have severe CKD will frequently be highly sensitive to the effects of thiazide diuretics. Thus, the addition of even low doses of a thiazide or thiazide-like diuretic often results in a dramatic increase in urinary salt and water excretion.

The WNK-SPAK/OSR1 Signaling Pathway and NCC

For thiazide-sensitive sodium chloride reabsorption to occur in the DCT, two processes must take place. First, a sufficient number of NCC cotransporters must be present at the DCT apical membrane, where they can come into contact with salt in the tubular lumen and facilitate NaCl cotransport. To get to the apical membrane, NCC must traffic there from a storage pool of vesicles present inside DCT cells (Figure 5). Second, cotransporters present at the DCT lumen must be switched on by phosphorylation. Two related serine threonine kinases, the Ste20-like proline-alanine rich kinase (SPAK) and oxidative stress responsive kinase 1 (OSR1), phosphorylate NCC directly (38). Both of these proteins transfer phosphate groups from ATP to serine and threonine residues present in the intracellular NCC amino terminus (Figure 5). By doing so, SPAK and OSR1 enhance the ability of the cotransporter to transport sodium and chloride, presumably by altering its protein conformation.

A family of proteins called With-No-Lysine [amino acid=K] kinases (WNKs) phosphorylates and activates SPAK and OSR1. They can also regulate NCC trafficking by separate mechanisms. These serine threonine kinases were identified as NCC regulators slightly over a decade ago through studies of a rare inherited disorder called Familial Hyperkalemic Hypertension (FHHt; also known as pseudohypoaldosteronism type 2 or Gordon syndrome) (39). FHHt is characterized by the unusual triad of hypertension, hyperkalemia, and normal GFR. Other features include metabolic acidosis and hypercalciuria. Thus, patients with FHHt have a phenotype that is essentially the mirror image of Gitelman syndrome. Moreover, FHHt is

exquisitely sensitive to thiazide diuretics, indicating that the disorder is primarily a syndrome of NCC overactivity (40). In 2001, mutations in two WNKs, WNK1 and WNK4, were identified as the cause of FHHt (41). Although mutations in WNKs were the first to be implicated in the pathogenesis of FHHt, they only account for a minority of families affected by the disorder. Most FHHt-affected individuals harbor mutations in two other genes: the E3 ubiquitin ligase *Cullin 3* (CUL3) and its adaptor, *Kelch-like-3* (KLHL3) (42,43). These two proteins participate in a protein complex that degrades WNK1 and WNK4. A very recent series of papers from several laboratories show that CUL3 covalently attaches ubiquitin molecules to WNK1 and WNK4, marking them for disposal (44–46). To do so, KLHL3 must be present, because it connects CUL3 to the WNKs (Figure 6A).

An elaborate interrelationship between WNK1, WNK4, and the KLHL3/CUL3 complex underlies the pathogenesis of FHHt. WNK1 and WNK4 regulate NCC through distinct mechanisms. According to current models, in the baseline state, where NCC is inactive and WNK4 kinase activity is switched off, WNK4 acts as an inhibitor, suppressing NCC trafficking to the cell surface (Figure 6A) (47–49). FHHt-causing WNK4 mutations are missense mutations that reduce WNK4 binding to KLHL3 (44,46). Total WNK4 protein expression, therefore, is increased in patients who have these mutations (Figure 6B). Disease-causing WNK4 mutants can phosphorylate SPAK and OSR1 (50), but many of them cannot block NCC plasma membrane trafficking (51,52). Thus, increased mutant WNK4 protein expression stimulates NCC phosphorylation and trafficking to the cell surface, resulting in NCC overactivation (Figure 6B) (44,46).

Like WNK4, the kinase active form of WNK1 phosphorylates and activates SPAK and OSR1 (53). In contrast to WNK4, however, WNK1 does not inhibit NCC trafficking. Rather, kinase-active WNK1 reverses the inhibitory effect of WNK4 on NCC traffic (Figure 6, A and C) (48,51). It likely mediates this reversal by binding to and phosphorylating WNK4 (54). Although these complexes cannot block NCC trafficking, they can still stimulate NCC phosphorylation. Thus, high levels of kinase-active WNK1 protein expression facilitate NCC trafficking to the cell surface and enhance NCC phosphorylation status through SPAK/OSR1. FHHt-causing mutations in WNK1 are intron deletions that do not alter the protein structure. Instead, these mutations increase total WNK1 mRNA and protein expression (41). The increased protein expression overrides ubiquitination and degradation by the KLHL3/CUL3 complex, and the net effect of the mutation is to increase total WNK1 kinase activity (55). Thus, in FHHt caused by mutations of WNK1, increased WNK1 protein expression should suppress the inhibitory effect of WNK4 on NCC traffic while stimulating SPAK and OSR1, causing NCC to be overactive (Figure 6C).

FHHt-associated mutations in KLHL3 reduce binding of the CUL3/KLHL3 complex to WNK1 and WNK4 (45) (Figure 6D). Thus, mutations in KLHL3 diminish the amount of CUL3-mediated WNK1 and WNK4 degradation (44–46). This increases WNK1 and WNK4 abundance, resulting in enhanced WNK-dependent signaling and NCC activation (Figure 6D). Mutations in CUL3 always cause

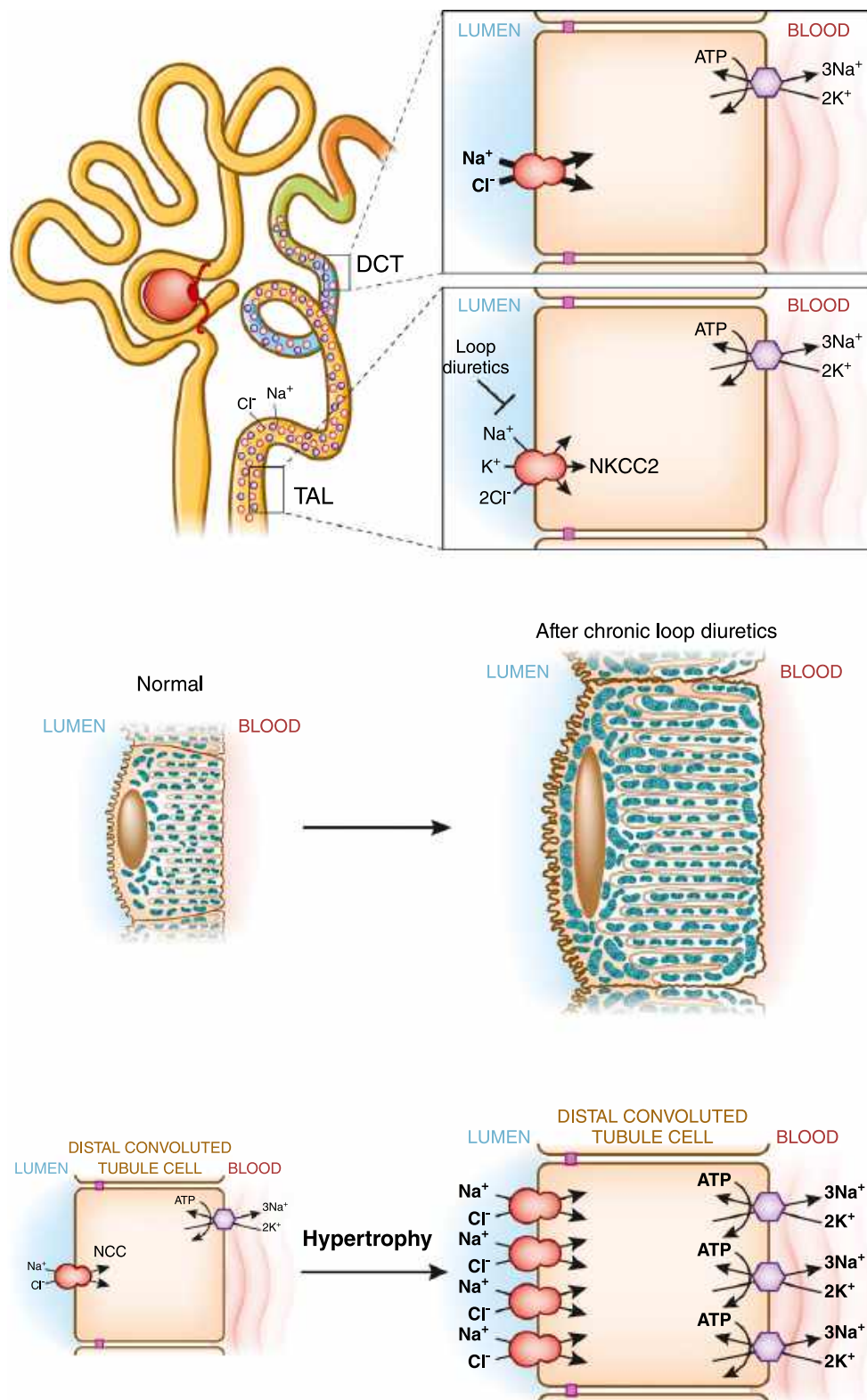


Figure 4. | Effect of chronic loop diuretic administration on DCT activity and morphology. (Upper panel) Sodium reabsorption normally mediated by the thick ascending limb Na - K - 2Cl cotransporter (NKCC2) is blocked by loop diuretics, such as furosemide and bumetanide. This results in enhanced Na^+ delivery to the DCT, which likely acts as a stimulus for DCT hypertrophy. (Lower panel) DCT hypertrophy manifests as an increase in mitochondrial size and basolateral membrane infoldings. The increase in NCC -mediated apical sodium transport coupled with enhanced activity of the sodium/potassium pump at the basolateral membrane results in a vectorial increase in sodium reabsorption and diuretic resistance.

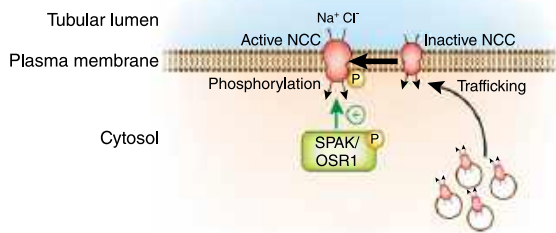


Figure 5. | SPAK and OSR1 phosphorylate and activate NCC. For NCC to be active, it must traffic to the plasma membrane from an intracellular storage pool. After it reaches the surface, it is in an inactive state until it is phosphorylated by two kinases, Ste20-like proline-alanine rich kinase (SPAK) and oxidative stress responsive kinase 1 (OSR1).

skipping of an exon, resulting in an in-frame deletion (42). It seems likely that this specific change in the CUL3 protein structure reduces WNK1 and WNK4 ubiquitination, but this remains to be tested.

Several clinically relevant hormones stimulate salt reabsorption in the distal nephron by increasing NCC activity, and in each of these cases, SPAK and/or OSR1 seem to be involved. These hormones include aldosterone (56,57), angiotensin II (58,59), insulin (60,61), and vasopressin (62,63). All of these hormones have been shown to increase the abundance of phosphorylated active NCC and, in some cases, have also been shown to increase NCC abundance or stimulate the movement of NCC from intracellular vesicles to the luminal membrane (64–66). Because the WNKs are the only known activators of SPAK and OSR1 in the kidney, these findings strongly suggest that hormones that regulate BP and extracellular fluid volume status recruit WNK-dependent signaling processes to activate NCC through SPAK and OSR1 (Figure 7). Indeed, recent work has shown, for example, that angiotensin II-mediated activation of NCC is a WNK4-dependent process (58). Defining the molecular details of how these physiologically relevant stimuli interface with the WNK-SPAK/OSR1 signaling pathway remains an active area of biomedical research.

Patients who are being treated with the immunosuppressant tacrolimus commonly present with hypertension, hyperkalemia, metabolic acidosis, and hypercalciuria—a clinical picture that in most respects resembles FHHt. Recently, it was shown that tacrolimus stimulates the activity of NCC, likely by enhancing its phosphorylation through the WNK-SPAK/OSR1 pathway (Figure 7) (67). Consistent with this finding, renal transplant patients on tacrolimus exhibited a greater urinary fractional excretion of chloride on bendroflumethiazide, indicating that tacrolimus-associated hypertension and hyperkalemia may be highly sensitive to thiazide diuretics (67). Larger clinical studies are currently underway to determine whether thiazides are superior to other agents as first-line therapy for the treatment of tacrolimus-associated hypertension.

K⁺ Secretion by the DCT

After reabsorption of K⁺ along the proximal tubule and loop of Henle, approximately 10% of filtered K⁺ reaches the DCT. As fluid travels down the DCT, the luminal potassium concentration increases, indicating that net K⁺ secretion occurs along the distal tubule. In the early DCT, the rate of K⁺ secretion is low, but it increases significantly in the late DCT (68–70). The enhanced K⁺ secretion parallels the progressive increase in lumen-negative transepithelial voltage observed in the late DCT. Indeed, a substantial component of the increased K⁺ secretion observed in this segment seems to be *voltage-dependent*. Voltage-dependent K⁺ secretion is mediated by the renal outer medullary potassium channel (ROMK; KCNJ1, also known as Kir1.1). ROMK-mediated K⁺ transport is dependent on the driving force for K⁺ secretion, which is primarily determined by electrogenic ENaC-mediated sodium reabsorption (71). Thus, as the activity of ENaC increases in the late DCT, more sodium is reabsorbed, which generates a lumen-negative stimulus for K⁺ efflux through ROMK potassium channels. As one can imagine, enhanced delivery of sodium ions to the late DCT and more downstream ENaC-expressing nephron segments—for example, through the use of loop diuretics—will enhance voltage-dependent K⁺ secretion.

The transepithelial voltage is not the only factor that determines the rate of K⁺ secretion in the DCT. Distal potassium secretion is also *flow-dependent*. Flow-dependent K⁺ secretion is mediated by the “big” or “maxi”-K⁺ channel (BK), a large conductance potassium channel that is expressed in all segments of the distal nephron, including the DCT, CNT, and collecting duct. During states of increased distal tubule flow, shear stress along the DCT activates the channel, possibly by increasing intracellular calcium levels and enhancing intracellular nitric oxide production (72,73). Both of the intracellular signals could increase the BK’s open probability and thereby enhance the rate of K⁺ efflux into the tubular lumen.

These observations have led to an updated model for K⁺ secretion in the distal nephron (Figure 8) (74,75). At baseline conditions, it has been proposed that the primary channel that mediates K⁺ secretion across the DCT lumen is ROMK. Under states of high urinary flow, such as during diuretic use, sodium delivery is enhanced, resulting in increased ENaC-mediated Na⁺ transport, depolarization of the apical membrane, and enhanced voltage-dependent K⁺ secretion through ROMK. In addition, the enhanced rate of urinary flow will trigger intracellular signaling mechanisms that result in enhanced BK activity. Thus, under low-flow conditions, ROMK is active, whereas under high-flow states, both ROMK and BK are active.

Additional K⁺ secretion occurs more distally in the connecting tubule and CCD (71). Classically, the collecting duct has been thought of as the primary nephron segment that mediates K⁺ secretion. This is perhaps owing to the ease with which collecting ducts can be accessed and studied in the laboratory. In contrast to the collecting duct, distal tubules are difficult to isolate and perfuse, and for years this technical problem may have caused physiologists to overlook the importance of the DCT in renal physiology. However, several studies indicate that the late DCT and CNT mediate a substantial fraction of total distal K⁺ secretion (71). Additional studies are needed to

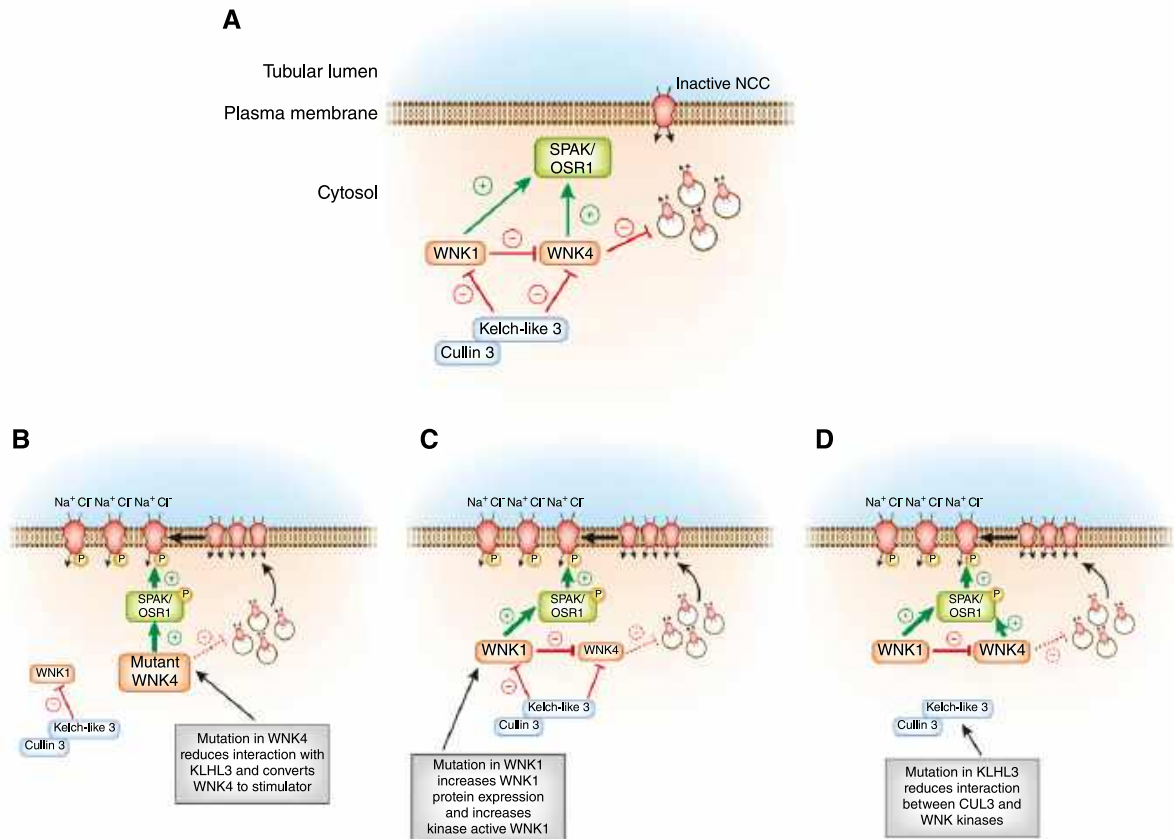


Figure 6. | A model of WNK-SPAK/OSR1 regulation of NCC and its role in the pathogenesis of Familial Hyperkalemic Hypertension (FHHt). (A) In the baseline inactive state, WNK4 suppresses NCC trafficking to the plasma membrane, holding the cotransporter in an intracellular storage pool. The kinase active form of WNK1 can reverse this process. The Kelch-like 3/Cullin-3 (KLHL3/CUL3) E3 ubiquitin ligase complex constitutively degrades the WNKs. (B) FHHt-associated mutations in WNK4 reduce binding to KLHL3, increasing WNK4 abundance and triggering NCC activation through the WNK effector kinases SPAK and OSR1. Additionally, FHHt-causing mutations in WNK4 reduce its inhibitory effect on NCC traffic (represented by the hatched bar-headed line), which releases NCC from its intracellular compartment, increasing its trafficking to the cell surface. Thus, FHHt mutations in WNK4 convert it into an NCC stimulator. (C) WNK1 gene mutations increase kinase-active WNK1 expression, which overcomes constitutive degradation by KLHL3/CUL3. Because kinase active WNK1 can inhibit wild-type WNK4 and activate SPAK/OSR1, increased WNK1 expression stimulates NCC surface delivery and phosphorylation. (D) Mutations in KLHL3 either reduce binding of KLHL3/CUL3 to WNK1 and WNK4 or disconnect CUL3 from KLHL3; in either case, the CUL3 E3 ligase is unable to mark WNK signaling complexes for degradation. Increased WNK1 and WNK4 abundance stimulates NCC trafficking to the surface and triggers NCC phosphorylation. FHHt-causing mutations in CUL3 also likely reduce its activity to WNKs, although the mechanism by which this occurs remains unknown. WNK, With-No-Lysine [amino acid=K] kinase.

determine the relative contribution of ROMK and BK in these nephron segments to whole-body potassium homeostasis.

Regulation of Distal Tubule Potassium Transport Regulation of ROMK Gating

ROMK is an “inward rectifier” potassium channel. This essentially means that the natural tendency of these channels is to transport potassium into cells rather than out of them. However, in the distal tubule, ROMK primarily functions to secrete potassium into the tubular lumen. The reason why ROMK can carry out such a function is because of differences in the chemical and electrical gradients in the DCT, CNT, and CCD. Potassium is the most abundant intracellular cation. Therefore, there is a chemical tendency for potassium to leak outward. Although the voltage on the interior aspect of the luminal membrane is negative, it is not

negative enough to counterbalance the high intracellular concentration of positively charged potassium cations. Therefore, K⁺ preferentially flows outward through ROMK. As described above, other channels that move positive charges inward (such as ENaC) will enhance this net outward flow of K⁺.

The capacity of a potassium channel to mediate inward rectification is modulated by a number of intrinsic factors, including the plasma membrane phosphoinositide 2 (76) and polyamines (77), but the most clinically relevant of these regulators of ROMK function is magnesium. Mg²⁺ binds to ROMK at a specific location on its cytoplasmic surface (78–80). By binding to ROMK at this site, Mg²⁺ blocks the channel’s pore from the inside and prevents K⁺ from being secreted. In the absence of adequate intracellular magnesium concentrations, potassium is more freely secreted into the tubular lumen (Figure 9). This phenomenon is currently believed to be an important reason

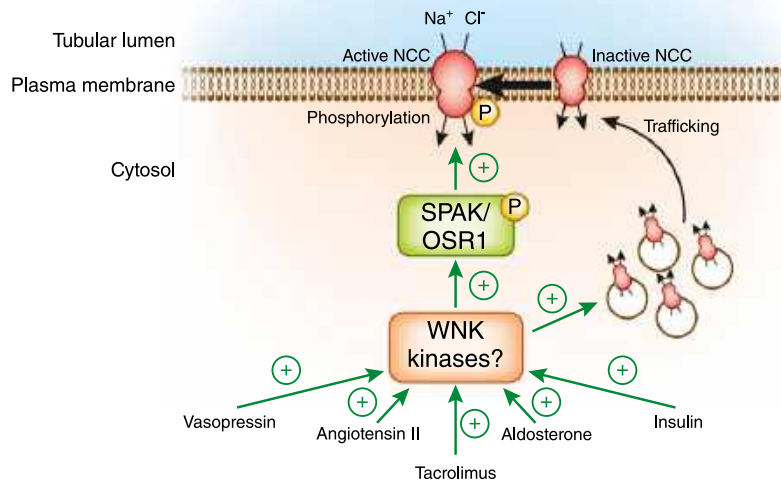


Figure 7. | A working model for NCC regulation through the WNK-SPAK/OSR1 signaling cascade. To date, a number of hormones have been shown to stimulate NCC phosphorylation at residues that are directly phosphorylated by SPAK and OSR1. These include aldosterone, angiotensin II, insulin, and vasopressin. Tacrolimus also enhances NCC phosphorylation at these sites, resulting in thiazide-sensitive NaCl reabsorption. In all cases, the mechanism likely involves hormonal activation of WNKs, which in turn, activates SPAK/OSR1. In some cases, such as aldosterone (66) and angiotensin II (58,64,65), hormone-induced changes in WNK-SPAK/OSR1-dependent signaling are also associated with increased trafficking of NCC to the plasma membrane.

why hypomagnesemia is often associated with enhanced distal K^+ secretion and refractory hypokalemia (81).

Aldosterone, Hyperkalemia, and Potassium Secretion

Hyperkalemia triggers aldosterone release from the adrenal gland. This results in an elevation of circulating aldosterone levels that enhances mineralocorticoid receptor-dependent signaling processes in the late DCT and downstream nephron segments. The mineralocorticoid receptor then triggers a signaling cascade that ultimately affects ROMK biogenesis. Specifically, the receptor translocates to the nucleus, where it stimulates the transcription of serum- and glucocorticoid-regulated kinase 1 (SGK1). SGK1 then phosphorylates a specific residue located in the cytoplasmic tail of ROMK; this event releases the channel from retention in the endoplasmic reticulum, allowing it to traffic more freely to the apical plasma membrane (82–84). Thus, through direct effects of SGK1 on ROMK traffic, aldosterone seems to ensure that an adequate number of potassium channels are available to facilitate K^+ secretion in the distal nephron. In addition to its effects on ROMK traffic, aldosterone also stimulates K^+ secretion by activating ENaC (85,86). As discussed above, the effect of this enhanced sodium transport through ENaC generates a lumen-negative transepithelial potential difference, which drives ROMK-mediated K^+ secretion (Figure 8).

The renal response to hyperkalemia seems to be much more than simply an aldosterone-dependent phenomenon. Potassium loading induced, for example, by administering a high K^+ diet rapidly increases the number of ROMK channels expressed at the plasma membrane of distal nephron cells within hours of treatment; this effect can be seen before the observed increase in circulating aldosterone levels,

suggesting that potassium has a direct effect on cells of the DCT. Similar effects have been noted with other components of the ion transport machinery expressed in the DCT, including the BK channel, the $Na^+-K^+-ATPase$, and ENaC (87–89). Recent evidence suggests that the aforementioned WNKs participate in the intracellular response of the DCT to hyperkalemia (90,91), and both WNK1 and WNK4 have been shown by several groups to influence the plasma membrane trafficking of both ROMK and BK channels *in vitro* (92–94). In fact, the bulk of evidence strongly suggests that the WNK signaling pathway seems to regulate transcellular sodium and potassium transport through completely different mechanisms, and it is this differential regulation of the two pathways that leads to the coexistence of hypertension and hyperkalemia seen in patients with FHHt (95). Although exciting progress has been made in this regard, additional studies are needed to define the *in vivo* relevance of these pathways to whole-body BP and potassium homeostasis in the general population.

Divalent Cation Reabsorption in the DCT

Although less is known about calcium and magnesium handling in the distal tubule, recent discoveries in the molecular mechanisms regulating these processes have expanded our understanding of how the DCT contributes to divalent cation homeostasis.

Calcium

Approximately 7%–10% of filtered calcium is reabsorbed in the DCT. In contrast to other segments of the nephron, which passively reabsorb calcium through paracellular routes, 100% of the calcium that is reabsorbed in the

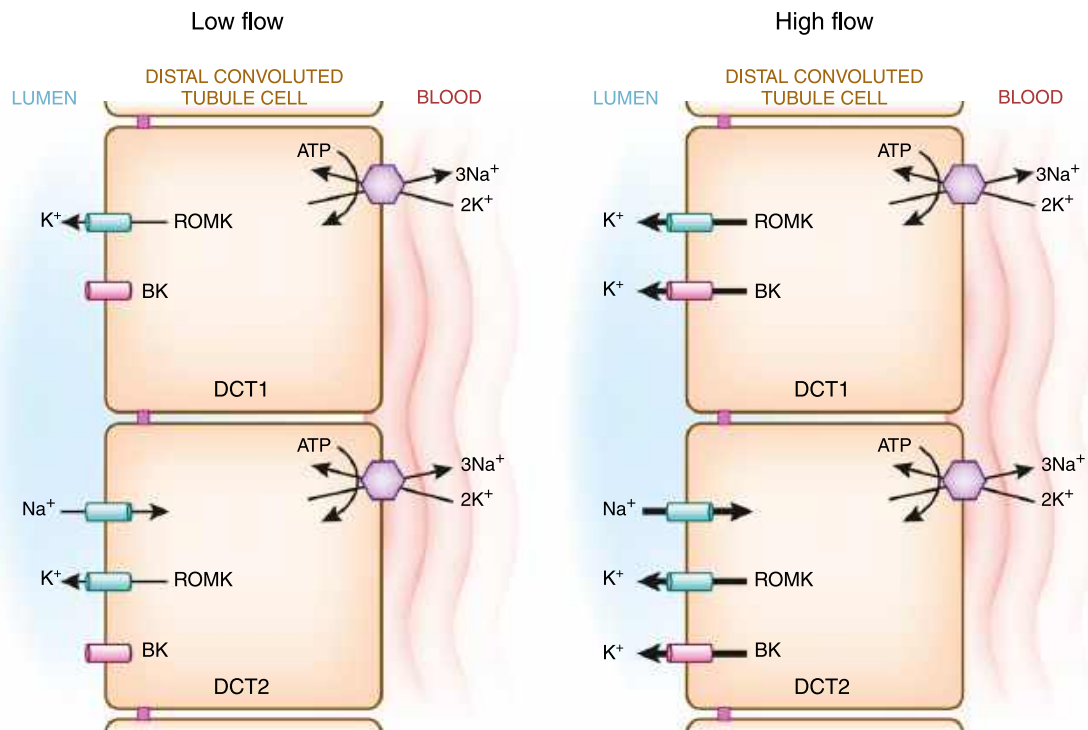


Figure 8. | Model of potassium secretion in the DCT. K^+ secretion in the distal nephron is voltage- and flow-dependent. In the low-flow state, “big”/“maxi”- K^+ channels (BK) are closed, and potassium secretion exclusively occurs through the renal outer medullary potassium channel (ROMK). ROMK is expressed in the early DCT, but its activity there is relatively low because of the transepithelial voltage, which is near zero. In the late DCT, ENaC-mediated Na^+ transport generates a driving force for ROMK-mediated K^+ secretion. During a high-flow state, both ROMKs and BKs mediate K^+ secretion. Increased sodium delivery and tubular fluid flow (for example, by infusing intravenous saline or Na bicarbonate or administering loop diuretics) increases ENaC activity, resulting in enhanced ROMK-mediated K^+ secretion. Additionally, the increased flow triggers an intracellular signaling mechanism in the DCT that opens BK channels, facilitating K^+ efflux into the tubular lumen.

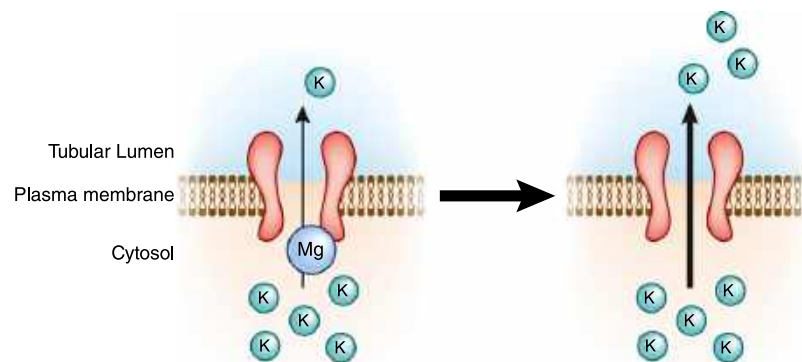


Figure 9. | Role of magnesium in ROMK potassium channel function. Potassium is the most abundant intracellular cation, creating a large chemical gradient that favors the outward flow of K^+ through ROMK. (Left panel) Normally, magnesium binds to a cytosol-exposed site in ROMK to limit this outward flow. (Right panel) During hypomagnesemia, fewer Mg^{2+} ions can bind to this site, and K^+ is secreted more freely. Thus, magnesium deficiency causes K^+ wasting. This likely explains why magnesium repletion is required to efficiently restore potassium concentrations to normal during concomitant hypomagnesemia and hypokalemia.

DCT occurs by active transcellular mechanisms. Apical calcium transport is mediated by the transient receptor potential channel subfamily V member 5 (TRPV5) (96) (Figure 10). On entry, Ca^{2+} associates with the calcium binding protein calbindin-D28K, which helps to buffer intracellular calcium levels and keep free calcium concentrations low (97) (Figure 10). After calcium is shuttled by

calbindin-D28K to the basolateral surface, Ca^{2+} is extruded into the peritubular fluid by a calcium ATPase and the Type 1 sodium calcium exchanger (NCX1; Figure 10).

Of these transport processes, apical calcium entry through TRPV5 seems to be the rate-limiting step for calcium reabsorption, and the activity of TRPV5 is regulated by

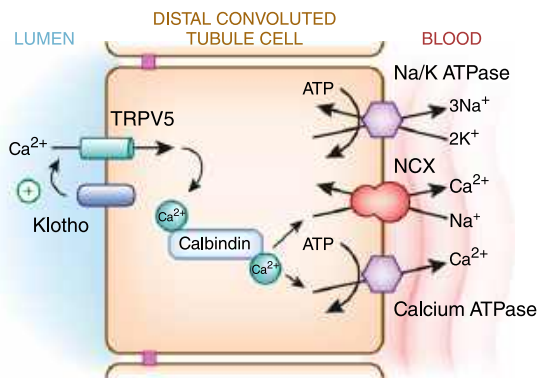


Figure 10. | Model of calcium reabsorption in the DCT. Apical calcium transport is mediated by transient receptor potential channel subfamily V member 5 (TRPV5) channels, which can be activated by the β -glucuronidase Klotho. Cytosolic calcium is immediately bound by calbindin-D28K, which shuttles calcium to the basolateral aspect of the DCT cell, where it can be transported out by the type 1 sodium calcium exchanger (NCX1) or calcium ATPases. These processes are tightly regulated by hormones, such as parathyroid hormone and 1,25-dihydroxyvitamin D (not shown).

several factors. Parathyroid hormone (PTH) affects TRPV5 channel activity through multiple mechanisms. PTH stimulates transcription of TRPV5 and prevents its degradation by inhibiting its removal from the apical surface of the DCT (98). In addition, PTH stimulates direct phosphorylation of TRPV5 through a protein kinase A-dependent signaling pathway, which alters the gating characteristics of the channel, increasing the likelihood that it will be open (99). 1,25-Dihydroxyvitamin D₃ also stimulates TRPV5 and calbindin-D28K expression. More recently, the protein Klotho was shown to regulate calcium homeostasis by increasing the cell surface expression of TRPV5. Klotho is a distal nephron-expressed transmembrane protein with β -glucuronidase enzyme activity. Klotho remodels sugars located on the extracellular loops of the TRPV5 molecule, which slows the rate of the channel's removal from the plasma membrane by enhancing binding to a secreted sugar binding protein, galectin-1 (100,101). Thus, by increasing the residence time of TRPV5 at the luminal membrane, Klotho increases TRPV5-mediated calcium reabsorption in the DCT (Figure 10). Interestingly, some evidence suggests that Klotho levels drop substantially during CKD (102), and Klotho-deficient mice develop several abnormalities of calcium homeostasis, including nephrocalcinosis, osteopenia, and hypercalciuria (103).

Hypercalcemia is a common side effect of thiazide diuretics (104). Because these drugs act on NCC-mediated salt transport in the DCT, one might assume that they somehow alter distal TRPV5-mediated calcium transport. Interestingly, this assumption turns out not to be the case. Knockout mice lacking TRPV5 are still capable of developing thiazide-induced hypocalciuria, which is probably because of the passive hyper-reabsorption of calcium with sodium and water in the proximal tubule (105) (Figure 11). Thiazide-associated volume depletion may trigger this increase in proximal calcium reabsorption.

Magnesium

The DCT reabsorbs about 10% of filtered magnesium and is the primary site of active transcellular Mg²⁺ reabsorption. Apical Mg²⁺ transport is mediated by transient receptor potential cation channel subfamily M member 6 (TRPM6), a voltage-driven divalent cation channel that is expressed in the early and late DCTs (106) (Figure 12). Like TRPV5, TRPM6 is a member of the transient receptor potential channel superfamily. TRPM6 has a 5-fold preference for magnesium ions over calcium ions, and these transport characteristics allow it to effectively function as a magnesium channel. Mutations in TRPM6 cause hypomagnesemia with secondary hypocalcemia, a rare Mendelian disorder of renal magnesium wasting.

Other than this fairly recently described magnesium transport pathway, relatively little is known about other transport processes that facilitate transcellular Mg²⁺ reabsorption. Unlike its fellow divalent cation calcium, evidence does not exist for an intracellular magnesium binding protein that buffers cytosolic magnesium concentrations. With regards to basolateral Mg²⁺ transport, a mechanism exists for the reclamation of magnesium back into the peritubular fluid and bloodstream; however, the precise molecular identities of these transport processes remain obscure. One putative candidate is cyclin M₂ (ancient conserved domain-containing protein 2), a distal nephron-expressed basolateral membrane protein that has been described by some as an Mg²⁺/metal ion transporter (107) and others as a magnesium sensor (108,109). Another candidate is the basolateral magnesium transporter SLC41A1, which is localized to the DCT. A loss-of-function mutation in this gene was recently shown to cause nephronophthisis, suggesting that it plays an important role in tubular function and morphology (110).

Emerging evidence gained from studies of families with rare inherited disorders of magnesium wasting suggests that the membrane voltage of the DCT plays a critical role in the control of magnesium reabsorption. In isolated dominant hypomagnesemia, mutations in the small γ -subunit of the Na⁺-K⁺-ATPase, FXD2, alter pump function (111–113). Specifically, the absence of this subunit, which is highly expressed in the thick ascending limb of Henle's loop and DCT, has been shown to alter the affinity of the Na⁺-K⁺-ATPase for sodium and potassium (114,115). FXD2 mutations reduce subunit binding to the pump, although it is still unclear how this reduced interaction causes hypomagnesemia. The aforementioned EAST/SeSAME syndrome (Figure 3) is another hypomagnesemic disorder, in which the basolateral membrane voltage is likely altered. In this case, inactivating mutations of Kir4.1 reduce K⁺ recycling across the basolateral membrane, which could result in reduced sodium pump function, alteration of the basolateral membrane potential, and diminished transcellular magnesium transport (26).

In 2009, a fascinating new Mendelian disorder was discovered, in which patients develop hypomagnesemia because of a presumed alteration in the apical membrane voltage of the DCT. This autosomal dominant form of hypomagnesemia was attributed to a mutation in the Shaker-related voltage-gated K⁺ channel Kv1.1 (116). This channel is exclusively expressed at the apical membrane of

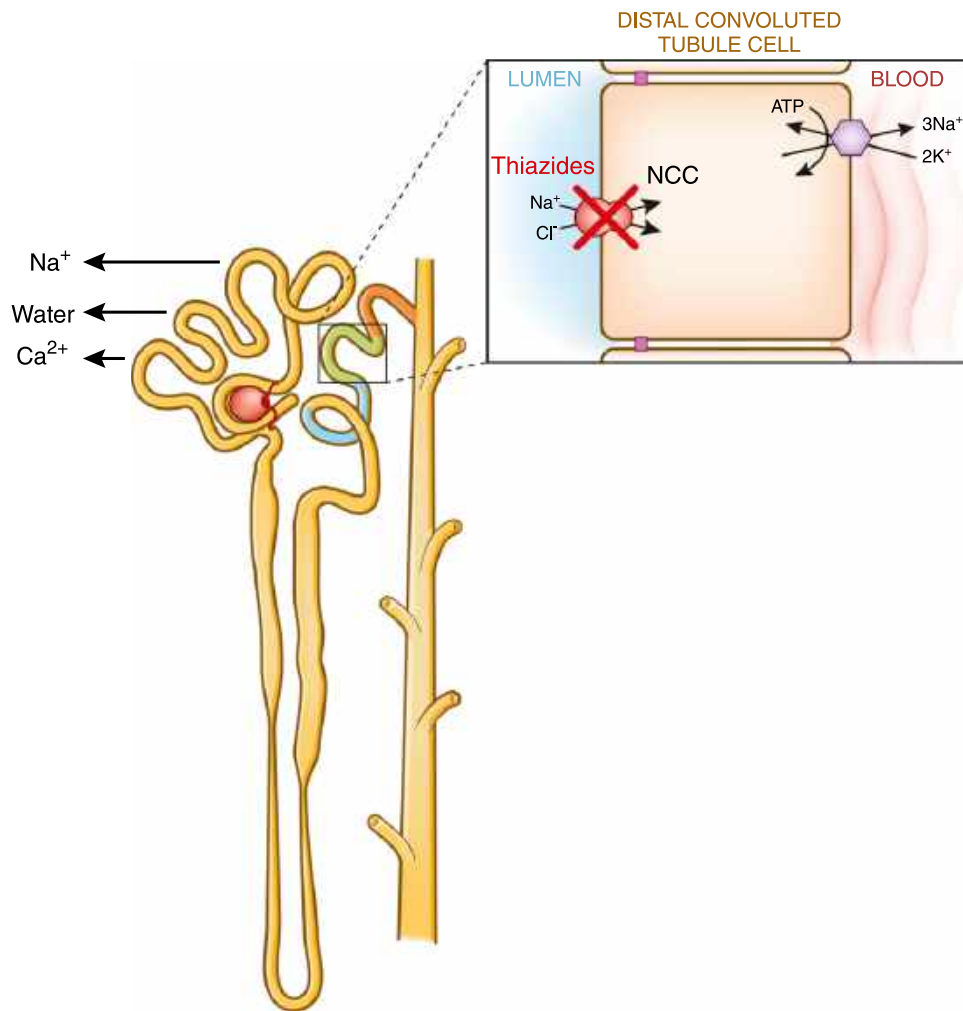


Figure 11. | Thiazide-induced hypocalciuria. Administration of thiazide diuretics reduces urinary calcium excretion and can sometimes cause hypercalcemia. The mechanism likely involves an increase in bulk calcium reabsorption with sodium and water in the proximal tubule. Thiazide-induced volume depletion might be the stimulus that triggers this process.

the early and late DCTs and secretes K^+ into the tubular lumen. It is believed that by secreting K^+ , Kv1.1 channels extrude positive charges into the lumen that provide a driving force for TRPM6-dependent Mg^{2+} transport (Figure 12). Mutation of Kv1.1 at a specific residue renders the channel nonfunctional, and one mutant allele is sufficient to cause the disease, presumably because of dominant negative inhibition of the remaining wild-type allele (117). A current theory is that the lack of functional Kv1.1 channels decreases the efflux of potassium cations into the lumen, which, in turn, would be expected to make the intracellular voltage on the luminal membrane more positive, decreasing the voltage gradient for luminal Mg^{2+} entry. Although this hypothesis is provocative, the pathophysiology may not be so simple: as discussed above in the section on ROMK gating, such a change in the luminal membrane potential would be expected to markedly enhance ROMK-mediated K^+ secretion and cause hypokalemia, which is not observed in the disorder. Perhaps compensatory changes in ROMK, BK, or even ENaC expression in more downstream nephron segments, where Kv1.1 is not expressed (such as the CNT and

CCD) (116), mitigate potassium losses, although to date, this hypothesis has not been tested.

EGF has emerged as an important regulator of Mg^{2+} handling in the DCT. In a Dutch family with a recessive form of selective hypomagnesemia, affected members had a mutation in the gene encoding the precursor form of EGF (118). This precursor molecule, pro-EGF, is a membrane protein that is expressed at the basolateral membrane of DCT cells. On arrival, the pro-EGF can be proteolytically cleaved to release soluble EGF, which then can interact with the EGF receptor and trigger a signaling cascade that stimulates TRPM6 (Figure 12). The hypomagnesemia-causing mutation results in missorting of pro-EGF, such that its delivery to the basolateral membrane of the DCT is inefficient. Ultimately, this impairs EGF-dependent signaling processes, including EGF-mediated stimulation of TRPM6 (119). The clinical importance of EGF-dependent regulation of TRPM6 is illustrated by the side effect profile of cetuximab, an EGF receptor antagonist used to treat colonic adenocarcinomas. Patients receiving systemic cetuximab can develop profound renal magnesium wasting because of the

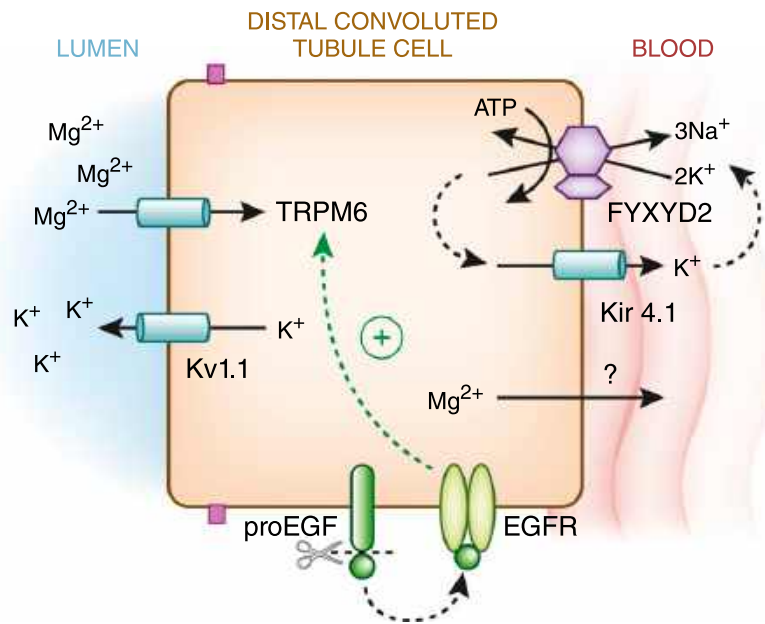


Figure 12. | Model of magnesium reabsorption in the DCT. The magnesium channel transient receptor potential cation channel subfamily M member 6 (TRPM6) mediates luminal magnesium entry. At the luminal membrane, the K^+ channel Kv1.1 extrudes K^+ ions into the tubular lumen; this process probably generates an electrical driving force for Mg^{2+} entry through TRPM6. TRPM6 activity is stimulated by the magnesiotropic hormone EGF, which triggers an intracellular signaling cascade in the DCT after cleavage from pro-EGF and binding to basolateral EGF receptors (EGFRs). After transluminal entry, cytosolic Mg^{2+} is then transported out of the basolateral side of the DCT through unclear mechanisms, although cyclin M_2 and SLC41A1 are candidate magnesium transport pathways that might mediate the process. Basolateral membrane voltage generated by the $Na^+-K^+-ATPase$ is critical for Mg^{2+} exit, which is illustrated by Kir4.1 mutations in EAST/SeSAME syndrome that reduce pump activity by impaired recycling (Figure 3) or small γ -subunit of the $Na^+-K^+-ATPase$ (FXND2) mutations, which alter the pump's affinity for Na^+ and K^+ .

inhibition of EGF-dependent stimulation of TRPM6 in the DCT.

Hypomagnesemia is a common side effect of thiazide diuretics. Thiazides sharply downregulate TRPM6 expression in the DCT (105), causing decreased magnesium reabsorption and hypomagnesemia. The mechanism by which TRPM6 expression is downregulated by thiazides remains unknown, although several mechanisms have been proposed (105). One attractive hypothesis is that thiazide-induced hypoplasia of DCT cells decreases TRPM6 protein abundance, reducing the total number of channels expressed at the luminal membrane.

Summary

The DCT is a short but critically important nephron segment. The DCT consists of two distinct subsegments; both subsegments are highly metabolically active and play key roles in sodium, potassium, and divalent cation homeostasis. Insights from genetic diseases of BP, potassium, and calcium and magnesium balance have expanded our knowledge of the molecular machinery that mediates these processes. Although it is already known that the DCT plays an important part in certain common pathophysiological states, such as diuretic resistance, the role of the DCT in many other clinically relevant disease states awaits additional investigation. Now that our understanding of the regulatory machinery of the DCT is more complete, such investigations can be pursued with the intent of understanding disease

pathogenesis and developing new strategies for the treatment of DCT-related disorders, such as hypertensive and/or edematous states, hyper- or hypokalemic tubulopathies, disorders of divalent ion balance, and nephrolithiasis.

Acknowledgments

This work was supported, in part, by the National Institutes of Health Grants R01-DK51496 (to D.H.E.), R01-DK095841 (to D.H.E.), and P30-DK079307 (to the Pittsburgh Center for Kidney Research), R01-DK098145 (to A.R.S.), and a Mid-Level Veterans Affairs Career Development Grant (to A.R.S.).

Disclosures

None.

References

- Chabardès D, Gagnan-Brunette M, Imbert-Teboul M, Gontcharevskaja O, Montégut M, Cluque A, Morel F: Adenylate cyclase responsiveness to hormones in various portions of the human nephron. *J Clin Invest* 65: 439–448, 1980
- Reilly RF, Ellison DH: Mammalian distal tubule: Physiology, pathophysiology, and molecular anatomy. *Physiol Rev* 80: 277–313, 2000
- Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM: Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science* 237: 268–275, 1987
- Bostanjoglo M, Reeves WB, Reilly RF, Velázquez H, Robertson N, Litwack G, Morsing P, Dørup J, Bachmann S, Ellison DH: 11 β -hydroxysteroid dehydrogenase, mineralocorticoid

- receptor, and thiazide-sensitive Na-Cl cotransporter expression by distal tubules. *J Am Soc Nephrol* 9: 1347–1358, 1998
5. Meneton P, Loffing J, Warnock DG: Sodium and potassium handling by the aldosterone-sensitive distal nephron: The pivotal role of the distal and connecting tubule. *Am J Physiol Renal Physiol* 287: F593–F601, 2004
 6. Kaissling B, Kriz W: Structural analysis of the rabbit kidney. *Adv Anat Embryol Cell Biol* 56: 1–123, 1979
 7. Dörup J: Ultrastructure of distal nephron cells in rat renal cortex. *J Ultrastruct Res* 92: 101–118, 1985
 8. Hierholzer K, Wiederholt M: Some aspects of distal tubular solute and water transport. *Kidney Int* 9: 198–213, 1976
 9. Levief F, Hübner CA, Houillier P, Morla L, El Moghrabi S, Brideau G, Hassan H, Parker MD, Kurth I, Kougoumtzes A, Sinning A, Pech V, Riemondy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, Eladari D: The Na⁺-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na⁺ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest* 120: 1627–1635, 2010
 10. Ellison DH, Velázquez H, Wright FS: Thiazide-sensitive sodium chloride cotransport in early distal tubule. *Am J Physiol* 253: F546–F554, 1987
 11. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Gitelman HJ, Lifton RP: Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet* 12: 24–30, 1996
 12. Kunchaparty S, Palcsó M, Berkman J, Velázquez H, Desir GV, Bernstein P, Reilly RF, Ellison DH: Defective processing and expression of thiazide-sensitive Na-Cl cotransporter as a cause of Gitelman's syndrome. *Am J Physiol* 277: F643–F649, 1999
 13. Donnelly BF, Needham PG, Snyder AC, Roy A, Khadem S, Brodsky JL, Subramanya AR: Hsp70 and Hsp90 multichaperone complexes sequentially regulate thiazide-sensitive cotransporter endoplasmic reticulum-associated degradation and biogenesis. *J Biol Chem* 288: 13124–13135, 2013
 14. Needham PG, Mikoluk K, Dhakarwal P, Khadem S, Snyder AC, Subramanya AR, Brodsky JL: The thiazide-sensitive NaCl cotransporter is targeted for chaperone-dependent endoplasmic reticulum-associated degradation. *J Biol Chem* 286: 43611–43621, 2011
 15. Loffing J, Zecevic M, Féraile E, Kaissling B, Asher C, Rossier BC, Firestone GL, Pearce D, Verrey F: Aldosterone induces rapid apical translocation of ENaC in early portion of renal collecting system: Possible role of SGK. *Am J Physiol Renal Physiol* 280: F675–F682, 2001
 16. Schmitt R, Ellison DH, Farman N, Rossier BC, Reilly RF, Reeves WB, Oberbäumer I, Tapp R, Bachmann S: Developmental expression of sodium entry pathways in rat nephron. *Am J Physiol* 276: F367–F381, 1999
 17. Wright FS: Increasing magnitude of electrical potential along the renal distal tubule. *Am J Physiol* 220: 624–638, 1971
 18. Hayslett JP, Boulpaep EL, Kashgarian M, Giebisch GH: Electrical characteristics of the mammalian distal tubule: Comparison of Ling-Gerard and macroelectrodes. *Kidney Int* 12: 324–331, 1977
 19. Velázquez H, Wright FS: Effects of diuretic drugs on Na, Cl, and K transport by rat renal distal tubule. *Am J Physiol* 250: F1013–F1023, 1986
 20. Yoshitomi K, Shimizu T, Taniguchi J, Imai M: Electrophysiological characterization of rabbit distal convoluted tubule cell. *Pflügers Arch* 414: 457–463, 1989
 21. Hamilton KL, Devor DC: Basolateral membrane K⁺ channels in renal epithelial cells. *Am J Physiol Renal Physiol* 302: F1069–F1081, 2012
 22. Reichold M, Zdebik AA, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeyer I, Penton D, Baukowitz T, Hulton SA, Witzgall R, Ben-Zeev B, Howie AJ, Kleta R, Bockenhauer D, Warth R: KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. *Proc Natl Acad Sci U S A* 107: 14490–14495, 2010
 23. Schultz SG: Homocellular regulatory mechanisms in sodium-transporting epithelia: Avoidance of extinction by “flush-through”. *Am J Physiol* 241: F579–F590, 1981
 24. Scholl UI, Choi M, Liu T, Ramaekers VT, Häusler MG, Grimmer J, Tobe SW, Farhi A, Nelson-Williams C, Lifton RP: Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. *Proc Natl Acad Sci U S A* 106: 5842–5847, 2009
 25. Bockenhauer D, Feather S, Stănescu HC, Bandulik S, Zdebik AA, Reichold M, Tobin J, Lieberer E, Sterner C, Landouere G, Arora R, Sirimanna T, Thompson D, Cross JH, van't Hoff W, Al Masri O, Tullus K, Yeung S, Anikster Y, Klootwijk E, Hubank M, Dillon MJ, Heitzmann D, Arcos-Burgos M, Knepper MA, Dobbie A, Gahl WA, Warth R, Sheridan E, Kleta R: Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. *N Engl J Med* 360: 1960–1970, 2009
 26. Bandulik S, Schmidt K, Bockenhauer D, Zdebik AA, Humberg E, Kleta R, Warth R, Reichold M: The salt-wasting phenotype of EAST syndrome, a disease with multifaceted symptoms linked to the KCNJ10 K⁺ channel. *Pflügers Arch* 461: 423–435, 2011
 27. Lourdel S, Paulais M, Cluzeaud F, Bens M, Tanemoto M, Kurachi Y, Vandewalle A, Teulon J: An inward rectifier K(+) channel at the basolateral membrane of the mouse distal convoluted tubule: Similarities with Kir4-Kir5.1 heteromeric channels. *J Physiol* 538: 391–404, 2002
 28. Balkovetz DF: Claudins at the gate: Determinants of renal epithelial tight junction paracellular permeability. *Am J Physiol Renal Physiol* 290: F572–F579, 2006
 29. Pacheco-Alvarez D, Cristóbal PS, Meade P, Moreno E, Vazquez N, Muñoz E, Díaz A, Juárez ME, Giménez I, Gamba G: The Na⁺:Cl⁻ cotransporter is activated and phosphorylated at the amino-terminal domain upon intracellular chloride depletion. *J Biol Chem* 281: 28755–28763, 2006
 30. Estévez R, Boettger T, Stein V, Birkenhäger R, Otto E, Hildebrandt F, Jentsch TJ: Barttin is a Cl⁻ channel beta-subunit crucial for renal Cl⁻ reabsorption and inner ear K⁺ secretion. *Nature* 414: 558–561, 2001
 31. Thakker RV: Chloride channels in renal disease. *Adv Nephrol Necker Hosp* 29: 289–298, 1999
 32. Velázquez H, Silva T: Cloning and localization of KCC4 in rabbit kidney: Expression in distal convoluted tubule. *Am J Physiol Renal Physiol* 285: F49–F58, 2003
 33. Mercado A, Song L, Vazquez N, Mount DB, Gamba G: Functional comparison of the K⁺-Cl⁻ cotransporters KCC1 and KCC4. *J Biol Chem* 275: 30326–30334, 2000
 34. Gottschalk CW, Mylle M: Evidence that the mammalian nephron functions as a countercurrent multiplier system. *Science* 128: 594, 1958
 35. Boettger T, Hübner CA, Maier H, Rust MB, Beck FX, Jentsch TJ: Deafness and renal tubular acidosis in mice lacking the K-Cl cotransporter Kcc4. *Nature* 416: 874–878, 2002
 36. Ellison DH, Velázquez H, Wright FS: Adaptation of the distal convoluted tubule of the rat. Structural and functional effects of dietary salt intake and chronic diuretic infusion. *J Clin Invest* 83: 113–126, 1989
 37. Ellison DH: Diuretic resistance: Physiology and therapeutics. *Semin Nephrol* 19: 581–597, 1999
 38. Richardson C, Rafiqi FH, Karlsson HK, Moleleki N, Vandewalle A, Campbell DG, Morrice NA, Alessi DR: Activation of the thiazide-sensitive Na⁺-Cl⁻ cotransporter by the WNK-regulated kinases SPAK and OSR1. *J Cell Sci* 121: 675–684, 2008
 39. Hadchouel J, Delaloy C, Fauré S, Achard JM, Jeunemaitre X: Familial hyperkalemic hypertension. *J Am Soc Nephrol* 17: 208–217, 2006
 40. Mayan H, Vered I, Mouallem M, Tzadok-Witkon M, Pauzner R, Farfel Z: Pseudohypoadosteronism type II: Marked sensitivity to thiazides, hypercalciuria, normomagnesemia, and low bone mineral density. *J Clin Endocrinol Metab* 87: 3248–3254, 2002
 41. Wilson FH, Disse-Nicodème S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinases. *Science* 293: 1107–1112, 2001
 42. Boyden LM, Choi M, Choate KA, Nelson-Williams CJ, Farhi A, Toka HR, Tikhonova IR, Björnson R, Mane SM, Colussi G, Lebel M, Gordon RD, Semmekrot BA, Poujol A, Välimäki MJ, De Ferrari ME, Sanjad SA, Gutkin M, Karet FE, Tucci JR, Stockigt JR,

- Keppeler-Noreuil KM, Porter CC, Anand SK, Whiteford ML, Davis ID, Dewar SB, Bettinelli A, Fadowski JJ, Belsha CW, Hunley TE, Nelson RD, Trachtman H, Cole TR, Pinsky M, Bockenhauer D, Shenoy M, Vaidyanathan P, Foreman JW, Rasoulpour M, Thameem F, Al-Shahroury HZ, Radhakrishnan J, Gharavi AG, Goilav B, Lifton RP: Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. *Nature* 482: 98–102, 2012
43. Louis-Dit-Picard H, Barc J, Trujillano D, Miserey-Lenkei S, Bouatia-Naji N, Pylypenko O, Beaurain G, Bonnefond A, Sand O, Simian C, Vidal-Petiot E, Soukaseum C, Mandet C, Broux F, Chabre O, Delahousse M, Esnault V, Fiquet B, Houillier P, Bagnis CI, Koenig J, Konrad M, Landais P, Mourani C, Niaudet P, Probst V, Thauvin C, Unwin RJ, Soroka SD, Ehret G, Ossowski S, Caulfield M, Bruneval P, Estivill X, Froguel P, Hadchouel J, Schott JJ, Jeunemaitre X; International Consortium for Blood Pressure (ICBP): KLHL3 mutations cause familial hyperkalemic hypertension by impairing ion transport in the distal nephron. *Nat Genet* 44: 456–460, S1–S3, 2012
 44. Shibata S, Zhang J, Puthumana J, Stone KL, Lifton RP: Kelch-like 3 and Cullin 3 regulate electrolyte homeostasis via ubiquitination and degradation of WNK4. *Proc Natl Acad Sci U S A* 110: 7838–7843, 2013
 45. Ohta A, Schumacher FR, Mehellou Y, Johnson C, Knebel A, Macartney TJ, Wood NT, Alessi DR, Kurz T: The CUL3-KLHL3 E3 ligase complex mutated in Gordon's hypertension syndrome interacts with and ubiquitylates WNK isoforms: Disease-causing mutations in KLHL3 and WNK4 disrupt interaction. *Biochem J* 451: 111–122, 2013
 46. Wakabayashi M, Mori T, Isobe K, Sahara E, Susa K, Araki Y, Chiga M, Kikuchi E, Nomura N, Mori Y, Matsuo H, Murata T, Nomura S, Asano T, Kawaguchi H, Nonoyama S, Rai T, Sasaki S, Uchida S: Impaired KLHL3-mediated ubiquitination of WNK4 causes human hypertension. *Cell Rep* 3: 858–868, 2013
 47. Subramanya AR, Liu J, Ellison DH, Wade JB, Welling PA: WNK4 diverts the thiazide-sensitive NaCl cotransporter to the lysosome and stimulates AP-3 interaction. *J Biol Chem* 284: 18471–18480, 2009
 48. Golbang AP, Cope G, Hamad A, Murthy M, Liu CH, Cuthbert AW, O'Shaughnessy KM: Regulation of the expression of the Na/Cl cotransporter by WNK4 and WNK1: Evidence that accelerated dynamin-dependent endocytosis is not involved. *Am J Physiol Renal Physiol* 291: F1369–F1376, 2006
 49. Cai H, Cebotaru V, Wang YH, Zhang XM, Cebotaru L, Guggino SE, Guggino WB: WNK4 kinase regulates surface expression of the human sodium chloride cotransporter in mammalian cells. *Kidney Int* 69: 2162–2170, 2006
 50. Yang SS, Morimoto T, Rai T, Chiga M, Sahara E, Ohno M, Uchida K, Lin SH, Moriguchi T, Shibuya H, Kondo Y, Sasaki S, Uchida S: Molecular pathogenesis of pseudohypoaldosteronism type II: Generation and analysis of a Wnk4(D561A/+) knockin mouse model. *Cell Metab* 5: 331–344, 2007
 51. Yang CL, Angell J, Mitchell R, Ellison DH: WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J Clin Invest* 111: 1039–1045, 2003
 52. Wilson FH, Kahle KT, Sabath E, Lalioti MD, Rapson AK, Hoover RS, Hebert SC, Gamba G, Lifton RP: Molecular pathogenesis of inherited hypertension with hyperkalemia: The Na-Cl cotransporter is inhibited by wild-type but not mutant WNK4. *Proc Natl Acad Sci U S A* 100: 680–684, 2003
 53. Vitari AC, Deak M, Morrice NA, Alessi DR: The WNK1 and WNK4 protein kinases that are mutated in Gordon's hypertension syndrome phosphorylate and activate SPAK and OSR1 protein kinases. *Biochem J* 391: 17–24, 2005
 54. Yang CL, Zhu X, Wang Z, Subramanya AR, Ellison DH: Mechanisms of WNK1 and WNK4 interaction in the regulation of thiazide-sensitive NaCl cotransport. *J Clin Invest* 115: 1379–1387, 2005
 55. Vidal-Petiot E, Elvira-Matlot E, Mutig K, Soukaseum C, Baudrie V, Wu S, Cheval L, Huc E, Cambillau M, Bachmann S, Doucet A, Jeunemaitre X, Hadchouel J: WNK1-related Familial Hyperkalemic Hypertension results from an increased expression of L-WNK1 specifically in the distal nephron. *Proc Natl Acad Sci U S A* 110: 14366–14371, 2013
 56. Kim GH, Masilamani S, Turner R, Mitchell C, Wade JB, Knepper MA: The thiazide-sensitive Na-Cl cotransporter is an aldosterone-induced protein. *Proc Natl Acad Sci U S A* 95: 14552–14557, 1998
 57. Chiga M, Rai T, Yang SS, Ohta A, Takizawa T, Sasaki S, Uchida S: Dietary salt regulates the phosphorylation of OSR1/SPAK kinases and the sodium chloride cotransporter through aldosterone. *Kidney Int* 74: 1403–1409, 2008
 58. Castañeda-Bueno M, Cervantes-Pérez LG, Vázquez N, Uribe N, Kantesaria S, Morla L, Bobadilla NA, Doucet A, Alessi DR, Gamba G: Activation of the renal Na⁺:Cl⁻ cotransporter by angiotensin II is a WNK4-dependent process. *Proc Natl Acad Sci U S A* 109: 7929–7934, 2012
 59. Gonzalez-Villalobos RA, Janjoulia T, Fletcher NK, Giani JF, Nguyen MT, Riquier-Brison AD, Seth DM, Fuchs S, Eladari D, Picard N, Bachmann S, Delpire E, Peti-Peterdi J, Navar LG, Bernstein KE, McDonough AA: The absence of intrarenal ACE protects against hypertension. *J Clin Invest* 123: 2011–2023, 2013
 60. Komers R, Rogers S, Oyama TT, Xu B, Yang CL, McCormick J, Ellison DH: Enhanced phosphorylation of Na⁺:Cl⁻ cotransporter in experimental metabolic syndrome: Role of insulin. *Clin Sci (Lond)* 123: 635–647, 2012
 61. Sahara E, Rai T, Yang SS, Ohta A, Naito S, Chiga M, Nomura N, Lin SH, Vandewalle A, Ohta E, Sasaki S, Uchida S: Acute insulin stimulation induces phosphorylation of the Na-Cl cotransporter in cultured distal mpkDCT cells and mouse kidney. *PLoS ONE* 6: e24277, 2011
 62. Saritas T, Borschewski A, McCormick JA, Paliege A, Dathe C, Uchida S, Terker A, Himmerkus N, Bleich M, Demarets S, Laghmani K, Delpire E, Ellison DH, Bachmann S, Mutig K: SPAK differentially mediates vasopressin effects on sodium cotransporters. *J Am Soc Nephrol* 24: 407–418, 2013
 63. Pedersen NB, Hofmeister MV, Rosenbaek LL, Nielsen J, Fenton RA: Vasopressin induces phosphorylation of the thiazide-sensitive sodium chloride cotransporter in the distal convoluted tubule. *Kidney Int* 78: 160–169, 2010
 64. Sandberg MB, Riquier AD, Pihakaski-Maunsbach K, McDonough AA, Maunsbach AB: ANG II provokes acute trafficking of distal tubule Na⁺:Cl⁻ cotransporter to apical membrane. *Am J Physiol Renal Physiol* 293: F662–F669, 2007
 65. San-Cristobal P, Pacheco-Alvarez D, Richardson C, Ring AM, Vazquez N, Rafiqi FH, Chari D, Kahle KT, Leng Q, Bobadilla NA, Hebert SC, Alessi DR, Lifton RP, Gamba G: Angiotensin II signaling increases activity of the renal Na-Cl cotransporter through a WNK4-SPAK-dependent pathway. *Proc Natl Acad Sci U S A* 106: 4384–4389, 2009
 66. Rozansky DJ, Cornwall T, Subramanya AR, Rogers S, Yang YF, David LL, Zhu X, Yang CL, Ellison DH: Aldosterone mediates activation of the thiazide-sensitive Na-Cl cotransporter through an SGK1 and WNK4 signaling pathway. *J Clin Invest* 119: 2601–2612, 2009
 67. Hoorn EJ, Walsh SB, McCormick JA, Fürstenberg A, Yang CL, Roeschel T, Paliege A, Howie AJ, Conley J, Bachmann S, Unwin RJ, Ellison DH: The calcineurin inhibitor tacrolimus activates the renal sodium chloride cotransporter to cause hypertension. *Nat Med* 17: 1304–1309, 2011
 68. Schnermann J, Steipe B, Briggs JP: In situ studies of distal convoluted tubule in rat. II. K secretion. *Am J Physiol* 252: F970–F976, 1987
 69. Stanton BA, Giebisch GH: Potassium transport by the renal distal tubule: Effects of potassium loading. *Am J Physiol* 243: F487–F493, 1982
 70. Wade JB, Fang L, Coleman RA, Liu J, Grimm PR, Wang T, Welling PA: Differential regulation of ROMK (Kir1.1) in distal nephron segments by dietary potassium. *Am J Physiol Renal Physiol* 300: F1385–F1393, 2011
 71. Giebisch G: Renal potassium transport: Mechanisms and regulation. *Am J Physiol* 274: F817–F833, 1998
 72. Liu W, Xu S, Woda C, Kim P, Weinbaum S, Satlin LM: Effect of flow and stretch on the [Ca²⁺]_i response of principal and intercalated cells in cortical collecting duct. *Am J Physiol Renal Physiol* 285: F998–F1012, 2003
 73. Kudlacek PE, Pluznick JL, Ma R, Padanilam B, Sansom SC: Role of hbeta1 in activation of human mesangial BK channels by cGMP kinase. *Am J Physiol Renal Physiol* 285: F289–F294, 2003

74. Holtzclaw JD, Grimm PR, Sansom SC: Role of BK channels in hypertension and potassium secretion. *Curr Opin Nephrol Hypertens* 20: 512–517, 2011
75. Sansom SC, Welling PA: Two channels for one job. *Kidney Int* 72: 529–530, 2007
76. Huang CL, Feng S, Hilgemann DW: Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by Gbetagamma. *Nature* 391: 803–806, 1998
77. Ficker E, Taglialatela M, Wible BA, Henley CM, Brown AM: Spermine and spermidine as gating molecules for inward rectifier K⁺ channels. *Science* 266: 1068–1072, 1994
78. Nichols CG, Ho K, Hebert S: Mg(2⁺)-dependent inward rectification of ROMK1 potassium channels expressed in *Xenopus* oocytes. *J Physiol* 476: 399–409, 1994
79. Lu Z, MacKinnon R: Electrostatic tuning of Mg2⁺ affinity in an inward-rectifier K⁺ channel. *Nature* 371: 243–246, 1994
80. Welling PA, Ho K: A comprehensive guide to the ROMK potassium channel: Form and function in health and disease. *Am J Physiol Renal Physiol* 297: F849–F863, 2009
81. Huang CL, Kuo E: Mechanism of hypokalemia in magnesium deficiency. *J Am Soc Nephrol* 18: 2649–2652, 2007
82. Yoo D, Fang L, Mason A, Kim BY, Welling PA: A phosphorylation-dependent export structure in ROMK (Kir 1.1) channel overrides an endoplasmic reticulum localization signal. *J Biol Chem* 280: 35281–35289, 2005
83. Yoo D, Kim BY, Campo C, Nance L, King A, Maouyo D, Welling PA: Cell surface expression of the ROMK (Kir 1.1) channel is regulated by the aldosterone-induced kinase, SGK-1, and protein kinase A. *J Biol Chem* 278: 23066–23075, 2003
84. O'Connell AD, Leng Q, Dong K, MacGregor GG, Giebisch G, Hebert SC: Phosphorylation-regulated endoplasmic reticulum retention signal in the renal outer-medullary K⁺ channel (ROMK). *Proc Natl Acad Sci U S A* 102: 9954–9959, 2005
85. Staub O, Dho S, Henry P, Correa J, Ishikawa T, McGlade J, Rotin D: WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na⁺ channel deleted in Liddle's syndrome. *EMBO J* 15: 2371–2380, 1996
86. Debonneville C, Flores SY, Kamynina E, Plant PJ, Tauxe C, Thomas MA, Münster C, Chraïbi A, Pratt JH, Horisberger JD, Pearce D, Löffing J, Staub O: Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. *EMBO J* 20: 7052–7059, 2001
87. Palmer LG, Antonian L, Frindt G: Regulation of apical K and Na channels and Na/K pumps in rat cortical collecting tubule by dietary K. *J Gen Physiol* 104: 693–710, 1994
88. Estilo G, Liu W, Pastor-Soler N, Mitchell P, Carattino MD, Kleyman TR, Satlin LM: Effect of aldosterone on BK channel expression in mammalian cortical collecting duct. *Am J Physiol Renal Physiol* 295: F780–F788, 2008
89. Silva P, Hayslett JP, Epstein FH: The role of Na-K-activated adenosine triphosphatase in potassium adaptation. Stimulation of enzymatic activity by potassium loading. *J Clin Invest* 52: 2665–2671, 1973
90. Cheng CJ, Baum M, Huang CL: Kidney-specific WNK1 regulates sodium reabsorption and potassium secretion in mouse cortical collecting duct. *Am J Physiol Renal Physiol* 304: F397–F402, 2013
91. Liu Z, Wang HR, Huang CL: Regulation of ROMK channel and K⁺ homeostasis by kidney-specific WNK1 kinase. *J Biol Chem* 284: 12198–12206, 2009
92. Lazrak A, Liu Z, Huang CL: Antagonistic regulation of ROMK by long and kidney-specific WNK1 isoforms. *Proc Natl Acad Sci U S A* 103: 1615–1620, 2006
93. Kahle KT, Wilson FH, Leng Q, Lalioti MD, O'Connell AD, Dong K, Rapson AK, MacGregor GG, Giebisch G, Hebert SC, Lifton RP: WNK4 regulates the balance between renal NaCl reabsorption and K⁺ secretion. *Nat Genet* 35: 372–376, 2003
94. Wade JB, Fang L, Liu J, Li D, Yang CL, Subramanya AR, Maouyo D, Mason A, Ellison DH, Welling PA: WNK1 kinase isoform switch regulates renal potassium excretion. *Proc Natl Acad Sci U S A* 103: 8558–8563, 2006
95. Welling PA, Chang YP, Delpire E, Wade JB: Multigene kinase network, kidney transport, and salt in essential hypertension. *Kidney Int* 77: 1063–1069, 2010
96. Hoenderop JG, van der Kemp AW, Hartog A, van de Graaf SF, van Os CH, Willems PH, Bindels RJ: Molecular identification of the apical Ca2⁺ channel in 1, 25-dihydroxyvitamin D3-responsive epithelia. *J Biol Chem* 274: 8375–8378, 1999
97. Hemmingsen C: Regulation of renal calbindin-D28K. *Pharmacol Toxicol* 87[Suppl 3]: 5–30, 2000
98. Cha SK, Wu T, Huang CL: Protein kinase C inhibits caveolae-mediated endocytosis of TRPV5. *Am J Physiol Renal Physiol* 294: F1212–F1221, 2008
99. de Groot T, Lee K, Langeslag M, Xi Q, Jalink K, Bindels RJ, Hoenderop JG: Parathyroid hormone activates TRPV5 via PKA-dependent phosphorylation. *J Am Soc Nephrol* 20: 1693–1704, 2009
100. Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG: The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science* 310: 490–493, 2005
101. Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro-O M, Huang CL: Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. *Proc Natl Acad Sci U S A* 105: 9805–9810, 2008
102. Hu MC, Kuro-o M, Moe OW: Klotho and chronic kidney disease. *Contrib Nephrol* 180: 47–63, 2013
103. Alexander RT, Woudenberg-Vrenken TE, Buurman J, Dijkman H, van der Eerden BC, van Leeuwen JP, Bindels RJ, Hoenderop JG: Klotho prevents renal calcium loss. *J Am Soc Nephrol* 20: 2371–2379, 2009
104. Duarte CG, Winnacker JL, Becker KL, Pace A: Thiazide-induced hypercalcemia. *N Engl J Med* 284: 828–830, 1971
105. Nijenhuis T, Vallon V, van der Kemp AW, Löffing J, Hoenderop JG, Bindels RJ: Enhanced passive Ca2⁺ reabsorption and reduced Mg2⁺ channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. *J Clin Invest* 115: 1651–1658, 2005
106. Schlingmann KP, Weber S, Peters M, Niemann Nejsum L, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M: Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet* 31: 166–170, 2002
107. Goytain A, Quamme GA: Functional characterization of ACDP2 (ancient conserved domain protein), a divalent metal transporter. *Physiol Genomics* 22: 382–389, 2005
108. Stuiver M, Lainez S, Will C, Terryn S, Günzel D, Debaix H, Sommer K, Kopplin K, Thumfart J, Kampik NB, Querfeld U, Willnow TE, Némec V, Wagner CA, Hoenderop JG, Devuyst O, Knoers NV, Bindels RJ, Meij IC, Müller D: CNNM2, encoding a basolateral protein required for renal Mg2⁺ handling, is mutated in dominant hypomagnesemia. *Am J Hum Genet* 88: 333–343, 2011
109. de Baaij JH, Stuiver M, Meij IC, Lainez S, Kopplin K, Venselaar H, Müller D, Bindels RJ, Hoenderop JG: Membrane topology and intracellular processing of cyclin M2 (CNNM2). *J Biol Chem* 287: 13644–13655, 2012
110. Hurd TW, Otto EA, Mishima E, Gee HY, Inoue H, Inazu M, Yamada H, Halbritter J, Seki G, Konishi M, Zhou W, Yamane T, Murakami S, Caridi G, Ghiggeri G, Abe T, Hildebrandt F: Mutation of the Mg2⁺ transporter SLC41A1 results in a nephronophthisis-like phenotype. *J Am Soc Nephrol* 24: 967–977, 2013
111. Kantorovich V, Adams JS, Gaines JE, Guo X, Pandian MR, Cohn DH, Rude RK: Genetic heterogeneity in familial renal magnesium wasting. *J Clin Endocrinol Metab* 87: 612–617, 2002
112. Meij IC, Koenderink JB, van Bokhoven H, Assink KF, Groenestege WT, de Pont JJ, Bindels RJ, Monnens LA, van den Heuvel LP, Knoers NV: Dominant isolated renal magnesium loss is caused by misrouting of the Na(+),K(+)-ATPase gamma-subunit. *Nat Genet* 26: 265–266, 2000
113. Cairo ER, Friedrich T, Swarts HG, Knoers NV, Bindels RJ, Monnens LA, Willems PH, De Pont JJ, Koenderink JB: Impaired routing of wild type FXD2 after oligomerisation with FXD2-G41R might explain the dominant nature of renal hypomagnesemia. *Biochim Biophys Acta* 1778: 398–404, 2008
114. Béguin P, Wang X, Firsov D, Puoti A, Claes D, Horisberger JD, Geering K: The gamma subunit is a specific component of the Na,K-ATPase and modulates its transport function. *EMBO J* 16: 4250–4260, 1997

115. Arystarkhova E, Wetzel RK, Asinovski NK, Sweadner KJ: The gamma subunit modulates Na(+) and K(+) affinity of the renal Na,K-ATPase. *J Biol Chem* 274: 33183–33185, 1999
116. Glaudemans B, van der Wijst J, Scola RH, Lorenzoni PJ, Heister A, van der Kemp AW, Knoers NV, Hoenderop JG, Bindels RJ: A missense mutation in the Kv1.1 voltage-gated potassium channel-encoding gene KCNA1 is linked to human autosomal dominant hypomagnesemia. *J Clin Invest* 119: 936–942, 2009
117. van der Wijst J, Glaudemans B, Venselaar H, Nair AV, Forst AL, Hoenderop JG, Bindels RJ: Functional analysis of the Kv1.1 N255D mutation associated with autosomal dominant hypomagnesemia. *J Biol Chem* 285: 171–178, 2010
118. Groenestege WM, Thébault S, van der Wijst J, van den Berg D, Janssen R, Tejpar S, van den Heuvel LP, van Cutsem E, Hoenderop JG, Knoers NV, Bindels RJ: Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest* 117: 2260–2267, 2007
119. Thebault S, Alexander RT, Tiel Groenestege WM, Hoenderop JG, Bindels RJ: EGF increases TRPM6 activity and surface expression. *J Am Soc Nephrol* 20: 78–85, 2009

Published online ahead of print. Publication date available at www.cjasn.org.



Collecting Duct Principal Cell Transport Processes and Their Regulation

David Pearce,* Rama Soundararajan,[†] Christiane Trimpert,[‡] Ossama B. Kashlan,[§] Peter M.T. Deen,[‡] and Donald E. Kohan^{||}

Abstract

The principal cell of the kidney collecting duct is one of the most highly regulated epithelial cell types in vertebrates. The effects of hormonal, autocrine, and paracrine factors to regulate principal cell transport processes are central to the maintenance of fluid and electrolyte balance in the face of wide variations in food and water intake. In marked contrast with the epithelial cells lining the proximal tubule, the collecting duct is electrically tight, and ion and osmotic gradients can be very high. The central role of principal cells in salt and water transport is reflected by their defining transporters—the epithelial Na⁺ channel (ENaC), the renal outer medullary K⁺ channel, and the aquaporin 2 (AQP2) water channel. The coordinated regulation of ENaC by aldosterone, and AQP2 by arginine vasopressin (AVP) in principal cells is essential for the control of plasma Na⁺ and K⁺ concentrations, extracellular fluid volume, and BP. In addition to these essential hormones, additional neuronal, physical, and chemical factors influence Na⁺, K⁺, and water homeostasis. Notably, a variety of secreted paracrine and autocrine agents such as bradykinin, ATP, endothelin, nitric oxide, and prostaglandin E₂ counterbalance and limit the natriuretic effects of aldosterone and the water-retaining effects of AVP. Considerable recent progress has improved our understanding of the transporters, receptors, second messengers, and signaling events that mediate principal cell responses to changing environments in health and disease. This review primarily addresses the structure and function of the key transporters and the complex interplay of regulatory factors that modulate principal cell ion and water transport.

Clin J Am Soc Nephrol 10: 135–146, 2015. doi: 10.2215/CJN.05760513

Introduction

The principal cell is one of two major epithelial cell types in what is often referred to as the aldosterone-sensitive distal nephron, comprising the connecting segment through the collecting duct. Ion and water transport in this part of the nephron are highly regulated by a wide variety of stimuli, including hormones, autocrine and paracrine factors, osmotic conditions, and physical factors. The principal cell is central to salt and water transport, as reflected by its defining transporters—the epithelial sodium channel (ENaC) and the aquaporin 2 (AQP2) water channel.

In humans, by the time tubular fluid reaches the aldosterone-sensitive distal nephron under physiologic conditions, virtually all amino acids, glucose, bicarbonate, and other nonwaste organic solutes have been removed and water volume has been reduced to approximately 10% of that of glomerular filtrate. Thus, the absolute level of transport of critical ions and water in this nephron segment is markedly lower than in most upstream segments; however, the variability of transport rates is markedly higher. Ion and osmotic gradients between the tubule lumen and the interstitium are also more variable, and are frequently much higher than in other regions. A future article in this series will address the integrated tubule physiology and compare characteristics of different segments. The coordinated regulation of ENaC by aldosterone, and AQP2 by arginine

vasopressin (AVP) in principal cells is essential for the control of plasma Na⁺ and K⁺ concentrations, extracellular fluid volume, and BP in most vertebrates (1). In addition to these essential hormones, other hormones, autacoids, and mechanical factors influence Na⁺, K⁺, and water homeostasis. This review primarily addresses the regulation of Na⁺, K⁺, and water transport in principal cells.

Control of Principal Cell Ion Transport

Figure 1 shows the integrated transport of Na⁺ and K⁺ in a typical principal cell, emphasizing the regulatory events that control ENaC (the principal pathway for apical Na⁺) and the renal outer medullary K⁺ (ROMK) channel (the principal pathway for apical exit of K⁺). Aldosterone is the primary hormonal regulator of both Na⁺ and K⁺ transport, as addressed further below. ENaC is the primary target of regulation, and its stimulation by aldosterone affects both Na⁺ reabsorption and K⁺ secretion. Cl[−] transport is not shown in Figure 1 because it is not a simple function of principal cells; rather, Cl[−] transport is a function of both principal and intercalated cells, as well as the paracellular pathway (2,3).

This review discusses the key Na⁺ and K⁺ pathways operative in principal cells and their regulation, and also describes Cl[−] transport.

*Division of Nephrology, Department of Medicine, University of California, San Francisco, California;

[†]Department of Translational Molecular Pathology, MD Anderson Cancer Center, Houston, Texas;

[‡]Department of Physiology, Radboud University Medical Center, Nijmegen, The Netherlands; [§]Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; and

^{||}Division of Nephrology, University of Utah Health Sciences Center, Salt Lake City, Utah

Correspondence:

Dr. David Pearce, Division of Nephrology, Department of Medicine, University of California, San Francisco, HSE 672, San Francisco, CA 94143. Email: dpearce@medsfgh.ucsf.edu

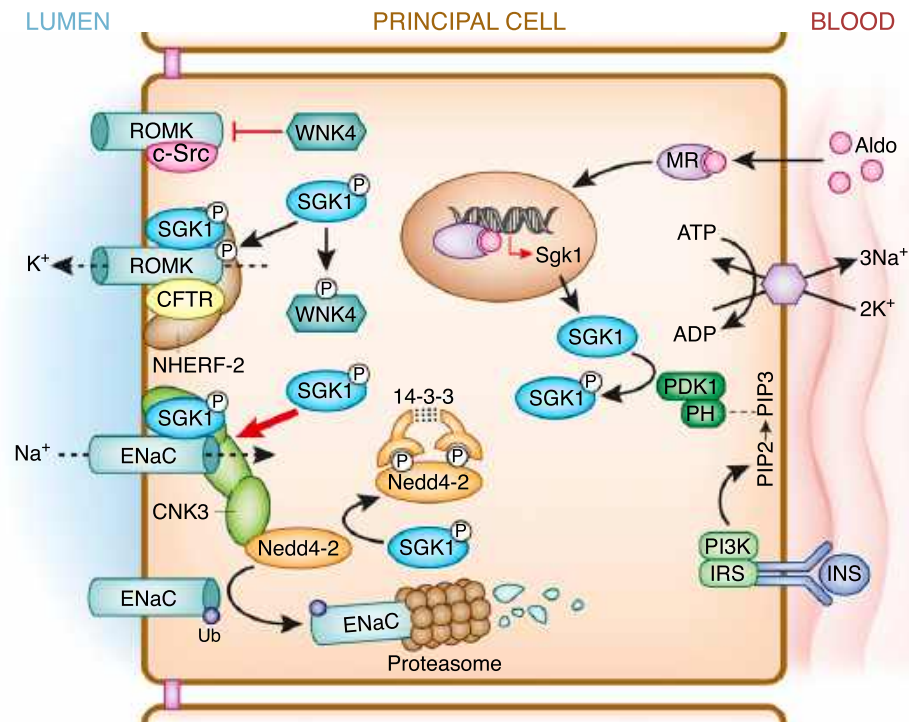


Figure 1. | Hormonal regulation of ion transport in a typical principal cell. Principal cells respond to a variety of stimuli to control Na⁺ and K⁺ transport. Aldosterone has the most pronounced effect. It acts through the MR to increase expression of the serine-threonine kinase SGK1. Other hormones, including insulin, regulate SGK1 activity through the master kinase phosphatidylinositol 3-kinase (PI3K). SGK1 phosphorylates a variety of proteins; Nedd4-2 is shown, which is an ENaC inhibitor (see the text). SGK1 phosphorylation triggers interaction of Nedd4-2 with 14-3-3 proteins, and hence inhibition, by reducing its internalization and degradation. ENaC surface expression and activity are governed by a multiprotein complex, whose assembly at the plasma membrane is orchestrated by the aldosterone-induced small chaperone GILZ (not shown), and the scaffold protein CNK3. Electrogenic Na⁺ reabsorption via ENaC is balanced by K⁺ secretion through ROMK and Cl⁻ reabsorption through multiple pathways (not shown). The apical surface expression and activity of ROMK are positively regulated by SGK1 through phosphorylation of WNK4. Similar to ENaC, ROMK assembly at the luminal membrane is dictated by a multiprotein complex facilitated by interactions with the scaffold proteins NHERF-1 and NHERF-2. The driving force that sets the electrochemical gradient for principal cell Na⁺ and K⁺ transport is the basolateral Na⁺-K⁺-ATPase. c-Src, cellular homologue of the v-src gene of the Rous sarcoma virus; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial Na⁺ channel; ERC, ENaC regulatory complex; INS, insulin; IRS, insulin receptor substrate; MR, mineralocorticoid receptor; Nedd4-2, neural precursor cell-expressed developmentally downregulated gene 4-2; PDK1, phosphoinositide-dependent kinase-1; PH, pleckstrin homology; ROMK, renal outer medullary K⁺; SGK1, serum- and glucocorticoid-regulated kinase 1; WNK, with no lysine kinase.

ENaC

Basic Biology of ENaC

ENaC mediates apical entry of Na⁺ into principal cells, and constitutes the rate-limiting step for transepithelial Na⁺ transport in the aldosterone-sensitive distal nephron. As is often the case for the rate-limiting step in any pathway, ENaC is also the primary locus of transepithelial Na⁺ transport regulation (Figure 1). Like all channels, it does not directly couple Na⁺ transport to the movement of any other ion or solute. Hence, unlike many upstream transporters such as the Na⁺-H⁺ exchanger isoform-3 (NHE3), Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2), and Na⁺-Cl⁻ cotransporter (NCC), it does not participate in secondary active transport (4). However, because ENaC mediates electrogenic Na⁺ transport, it increases the driving force for K⁺ secretion via K⁺ channels, such as ROMK (expressed in principal cells, see below) (5) and BK channels (expressed in both principal and intercalated cells). It also enhances H⁺ secretion by adjacent intercalated cells, as well as Cl⁻ reabsorption via a variety of pathways; a

future review in this series will address these topics, along with BK channels, in detail.

ENaC comprises three distinct, but structurally related, subunits (α , β , and γ) (6). Based on the homotrimeric subunit arrangement in the crystal structure of the acid-sensing ion channel (7), an ENaC relative, it is currently thought that ENaC is a heterotrimer (8). Although this issue is not fully resolved, considerable insight into ENaC function was gained by performing a comparison with the acid-sensing ion channel (Figure 2). ENaC is highly selective for Na⁺ (and Li⁺) over other ions (most notably K⁺), and is highly sensitive to the K⁺-sparing diuretic amiloride. Selectivity is mediated by a signature Gly/Ser-X-Ser sequence, which is adjacent to the amiloride binding site (Figure 2D). ENaC activity and cell surface expression are regulated by a variety of hormonal, autocrine, paracrine, and nonhormonal signals. These regulatory signals are integrated to produce appropriate levels of Na⁺ transport to meet physiologic demands. Recent evidence suggests that this signal integration requires ENaC at

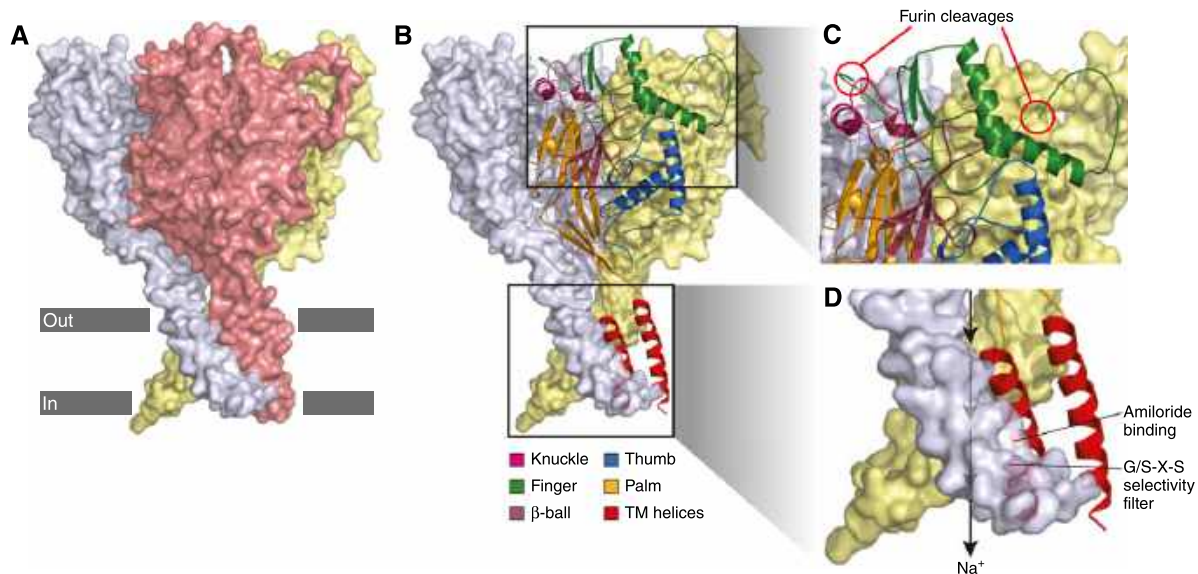


Figure 2. | Structural model of the ENaC extracellular domains and pore. The model represents a hypothetical α subunit trimer and was built on the basis of sequence homology to ASIC1 and functional data (8,122). Sequence conservation among ENaC subunits suggests that the α , β , and γ subunits adopt similar folds. The intracellular domains, accounting for 25%–30% of each subunit's mass, are absent from current models of ENaC structure. These domains contain essential sites for regulatory interactions (e.g., Nedd4-2 and CNK3) and modification (e.g., ubiquitinylation and phosphorylation). (A) Surface representation showing the spatial arrangement of the three subunits with the approximate location of outer and inner borders of the lipid bilayer. (B) One subunit is represented as a ribbon diagram showing the five extracellular domains and transmembrane α -helices labeled as indicated. (C) Close-up of the finger domain (from the model in B), highlighting the peripheral location of the furin cleavage sites. (D) Close-up of the pore highlighting the likely permeation pathway as well as sites implicated in amiloride binding and permeant ion discrimination. ASIC, acid-sensing ion channel; TM, transmembrane.

the plasma membrane to be organized into a large (1.2 MDa) multiprotein termed the ENaC regulatory complex (ERC), which includes both positive (e.g., serum- and glucocorticoid-regulated kinase 1 [SGK1]) and negative (e.g., neural precursor cell-expressed developmentally downregulated gene 4-2 [Nedd4-2]) regulators. Regulatory molecules within the ERC interact with the cytoplasmic domains of ENaC, which are absent in current models of the ENaC structure (Figure 2). The formation and stability of the complex requires an aldosterone-induced chaperone (GILZ1) and a scaffold protein (CNK3) (9,10), which keep the complex together by stimulating interactions among multiple proteins (Figure 1). It is interesting to note that CNK3, like many scaffolds involved in stabilizing membrane expression of transport proteins, has a PDZ (PSD-95/DLG-1/ZO-1) domain (1). ROMK membrane stability requires another PDZ domain protein, sodium-proton exchanger regulatory factor (NHERF) (both isoforms NHERF-1 and NHERF-2 have been implicated) (11).

Although the stable presence of ENaC at the apical membrane requires the ERC, its activity at the cell surface requires proteolytic cleavage at specific sites within the extracellular loops of the α and γ subunits to liberate embedded inhibitory tracts (12) (Figure 2). Under physiologic conditions, this effect appears to be mediated by furin and a secondary membrane-resident protease. Furin is a proprotein convertase that resides primarily in the trans-Golgi network and processes proteins transiting through the biosynthetic pathway. Furin increases ENaC open probability (i.e., the percentage of time the channel spends open) and, hence, net Na^+ transport. It is unclear at this time whether

the furin-mediated cleavage is an important locus of regulation or is a device to keep the channel from being turned on prematurely (see below). However, it is increasingly clear that ENaC cleavage plays a pathophysiologic role in the Na^+ retention associated with the nephrotic syndrome. The serine protease plasmin cleaves the ENaC γ subunit and activates the channel (13). Plasmin is not present in the tubule lumen under normal conditions; however, in the setting of proteinuria (as seen in the nephrotic syndrome), plasminogen is filtered by the glomerulus and can be converted to plasmin by urokinase, which is present within the tubular lumen (13). In the context of glomerular proteinuria, plasmin-dependent ENaC activation may contribute to Na^+ retention, and edema or hypertension (14).

Animals or humans with decreased ENaC function have severe disorders of Na^+ wasting and K^+ retention. Increased channel activity (or excess aldosterone) results in hypertension and K^+ wasting (15), as seen with the heritable disorder Liddle's syndrome. The first identified Liddle mutation resulted in a premature translation stop in the β subunit (16), leaving the Na^+ pore intact but deleting intracellular target sites for inhibitory control mechanisms (16). Other mutations that cause variable degrees of hyperactivation of the channel were also identified. On the basis of these observations, it was suggested that mild increases in ENaC activity could act in concert with other signaling defects in the pathogenesis of essential hypertension (17).

Hormonal Regulation of ENaC

Renin-Angiotensin-Aldosterone System. Aldosterone is central to the normal regulation of Na^+ and K^+ handling by

principal cells, and, hence, to the control of ion concentrations, extracellular fluid volume, and BP in all land mammals (18). The effects of aldosterone on ion transport in principal cells are mediated by the mineralocorticoid receptor (MR). The MR, which is almost certainly the only receptor for aldosterone in principal cells, is an intracellular hormone-regulated transcription factor that triggers coordinated changes in the expression of numerous genes. The key ones required for regulating Na^+ transport encode either transporters themselves, or regulatory proteins that control transporter abundance or activity. The bulk of evidence supports the view that the central effect of aldosterone is to increase ENaC apical membrane density approximately 2- to 5-fold (18,19) (Figure 1), but effects on channel open probability may also contribute. The ENaC α subunit gene itself is an important target; however, its transcription is stimulated somewhat slowly (over approximately 6 hours). Similarly, aldosterone stimulates transcription of both the α and β subunits of the Na^+ - K^+ -ATPase, but also relatively slowly (20) (see below). In fact, most of the aldosterone-induced increase in Na^+ transport occurs within the first 3 hours and is primarily mediated by rapidly stimulated genes that encode regulatory proteins, not by increases in transporter gene expression *per se*.

The best characterized of the ENaC regulatory proteins is the serine-threonine kinase SGK1 (21). Aldosterone acts through the MR to rapidly increase SGK1 gene transcription and, thus, the SGK1 protein level (Figure 1). Importantly, SGK1 must also be activated by two phosphorylation events that control its inherent activity (22). These activating phosphorylations are regulated by other hormones, including insulin and possibly angiotensin II (AngII) (23). Activated SGK1 then phosphorylates and regulates various targets, most notably the ubiquitin ligase Nedd4-2 (24), which post-translationally modifies proteins by covalently adding a ubiquitin group to specific lysines. Nedd4-2 inhibits ENaC by ubiquitinating the channel, triggering its internalization from the plasma membrane and ultimately its degradation (25). SGK1 phosphorylates and inhibits Nedd4-2, and this double negative (inhibiting the inhibitor) results in ENaC accumulation at the apical plasma membrane (26). Importantly, the above-mentioned Liddle mutation disrupts interaction between Nedd4-2 and ENaC, as if aldosterone were always present (18). In fact, aldosterone levels are suppressed in Liddle's syndrome, and hence it is a form of pseudohyperaldosteronism. It is also interesting to note that SGK1 indirectly regulates ENaC gene expression through effects on the activities of Dot1a and Af9 (27).

SGK1 acts in other cell types to regulate a variety of other transporters, including NHE3 (proximal tubule and thick ascending limb), NKCC2 (thick ascending limb), and NCC (distal convoluted tubule). It is particularly worth noting recent evidence supporting the idea that SGK1 regulation of NCC in distal convoluted tubule proceeds, at least in part, through regulation of Nedd4-2, in a manner similar to how the SGK1-Nedd4-2 module regulates ENaC in principal cells (28). On the other hand, SGK1 also regulates ROMK in principal cells (29), although this effect is likely less important than its effects on ENaC (see below). Small molecule inhibitors of SGK1 have been shown to have antihypertensive effects in animals; however, there have been no clinical trials (30). An area of considerable recent

interest is the convergent regulation by AngII and aldosterone of ENaC and other transporters such as NCC (31).

Atrial Natriuretic Peptide. Maintaining cardiorenal homeostasis by regulating fluid volume is an important aspect of the cardioprotective properties of atrial natriuretic peptide (ANP). ANP achieves these effects by regulating multiple renal processes, as will be addressed in other reviews in this series. It acts through the membrane-bound natriuretic peptide receptor-A, which triggers generation of the second messenger cGMP (32) to stimulate a variety of downstream targets including cGMP-dependent protein kinases and cGMP-gated ion channels (33). In principal cells, ANP inhibits ENaC, which contributes to its natriuretic properties (34). It also regulates BP by inhibiting renin secretion (35) and aldosterone production from the adrenal gland by downregulating the steroidogenic acute regulatory protein (36).

Insulin. The physiologic role for insulin in the control of Na^+ transport has remained obscure; however, its pathophysiologic effects are clear. Kidney tubule Na^+ transport remains insulin sensitive, even as other tissues and processes become resistant (37). Insulin stimulates NHE3-dependent Na^+ transport in the proximal tubule (38), whereas it stimulates ENaC in principal cells. As insulin levels rise to maintain normal glucose concentration, the retained sensitivity of Na^+ transport results in excessive Na^+ reabsorption in insulin-resistant states. Because neither Na^+ nor BP participates in the feedback loop to inhibit islet β -cell insulin secretion, the principal cell becomes an "unwilling accomplice" in the ensuing salt-sensitive hypertension (39). Further contributing to this problem, the vasodilatory effects of insulin are blunted in insulin-resistant states, and hence only the prohypertensive effects remain (40).

AVP. In addition to its critical role in regulating principal cell water transport (see below), AVP also has important effects to stimulate ENaC. These effects are mediated substantially by cAMP, which activates cAMP-dependent kinase (protein kinase A [PKA]). PKA can phosphorylate and inhibit Nedd4-2 in a manner similar to that of SGK1 (41,42). This direct effect is complicated by variable effects of AVP on the renin-angiotensin-aldosterone system (RAAS) hormonal axis, either to stimulate or suppress renin, depending on extracellular fluid volume status and AVP blood level (42). Importantly, despite stimulation of ENaC (which itself should raise the serum Na^+ concentration), the net effect of AVP is to lower Na^+ concentration. However, under conditions of high sodium consumption and low water intake (raising AVP levels, while lowering aldosterone), AVP may contribute to salt-sensitive hypertension through its effects to stimulate ENaC. It was suggested that AVP stimulation of ENaC might play a physiologically beneficial role in overall water conservation when both water and salt intake are low, although this effect would blunt its ability to lower serum Na^+ concentration (42). This suggestion harkens back to the clinical maxim that "the body defends volume above all else."

Paracrine and Autocrine Regulation of ENaC and Na^+ Transport in the Collecting Duct

Hormones are key regulators of principal cell Na^+ transport; however, another key aspect of this regulation involves local factors. In particular, autocrine (acting on the same cell)

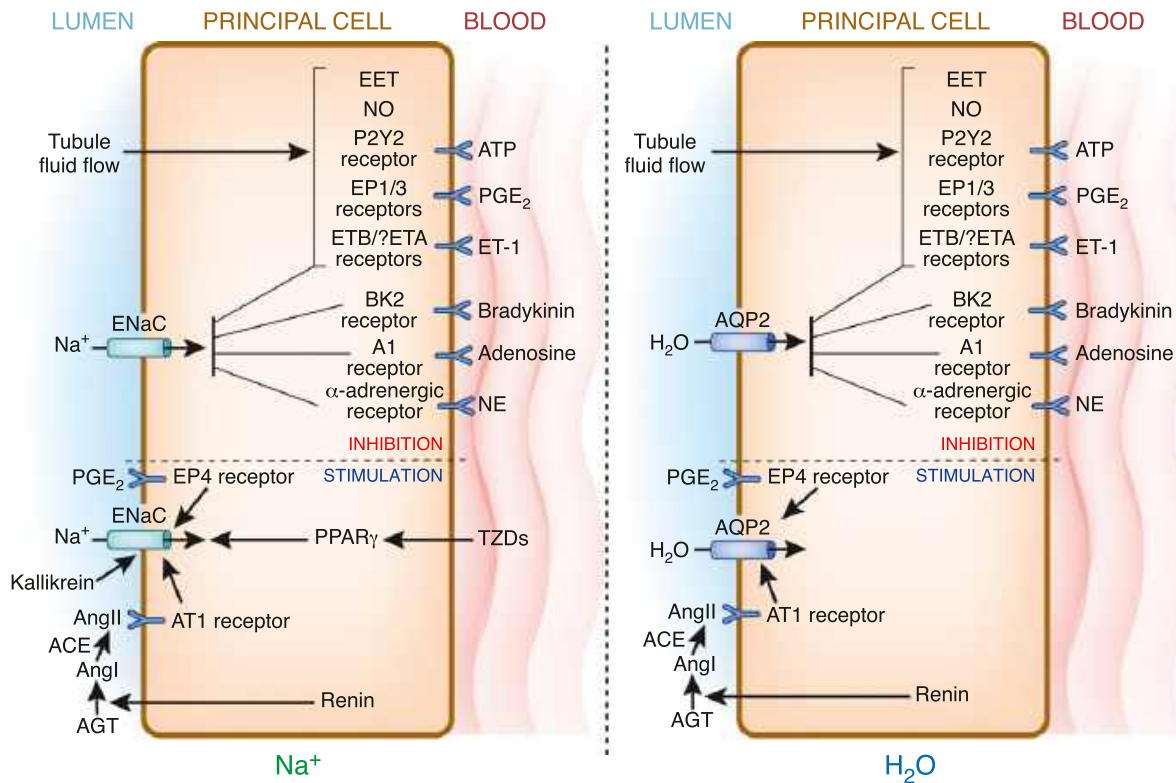


Figure 3. | Autocrine and paracrine regulation of collecting duct principal cell ENaC and AQP2. Much commonality exists in regulation of ENaC (left) and AQP2 (right). Flow stimulates ATP, PGE₂, and ET-1, which act on their cognate receptors to inhibit Na and water reabsorption. Similarly, bradykinin, adenosine, and NE act on their receptors to inhibit ENaC and AQP2. Flow-stimulated EET uniquely inhibits Na, but not water, transport. Compared with the wide variety of inhibitors, relatively few autocrine or paracrine factors stimulate ENaC and/or AQP2 activity. Renin, ultimately via AngII, as well as PGE₂ binding to EP4 receptors, are potentially capable of augmenting principal cell Na and water transport. TZDs (via PPAR γ) and kallikrein (via cleavage of an autoinhibitory domain in ENaC) may increase Na reabsorption. See the text for more detailed descriptions of each regulatory factor. ACE, angiotensin-converting enzyme; AGT, angiotensinogen; Ang, angiotensin; AQP, aquaporin; EET, eicosatetraenoic acid; EP, PGE receptor; ET, endothelin; NE, norepinephrine; NO, nitric oxide; PPAR γ , peroxisome proliferator-activated receptor- γ ; TZD, thiazolidinedione.

and paracrine (acting on neighboring cells) factors play an important role in the modulation of principal cell Na⁺ transport. Physical factors, such as tubule fluid flow, can also play a role. Figure 3 summarizes these effects.

Adrenergic Nerves. The findings of close apposition of sympathetic nerve extensions and principal cells (43), as well as collecting duct expression of β - and α -adrenergic receptors, suggests that principal cell function can be modulated by catecholamines released by efferent renal sympathetic nerves. The nature of such regulation is unclear because differing results have been obtained depending upon the species studied and the agonist utilized. It is likely that adrenergic effects on principal cells are complex, depending upon whether β - or α -adrenergic receptors are activated, the specific cAMP-regulated pathway affected (urea, Na, or water), and the hormonal milieu. In general, α -adrenergic receptor activation in the collecting duct appears to inhibit agonist-induced cAMP accumulation (43,44); however, much more clarification is needed.

Prostaglandins and Cytochrome P450 Metabolites. AA in the collecting duct can be metabolized by cyclooxygenases to prostaglandins (PGs) and by cytochrome P450 epoxygenase to form various epoxyeicosatrienoic acids (EETs). The collecting duct produces relatively large amounts of PGs and particularly PGE₂ (45,46). The collecting duct expresses three

PG receptors (EP1, EP3, and EP4). Collecting duct PGE₂ production is stimulated by a variety of factors that generally exert natriuretic and diuretic effects, including ATP, EETs, endothelin (ET)-1, shear stress, and others (45,47). The effects of PGE₂ on collecting duct Na⁺ transport are complex. In the absence of agonists, PGE₂ may augment cAMP-dependent Na⁺ reabsorption in the collecting duct, most likely through stimulation of EP4 receptors. However, in the presence of agonists, PGE₂ inhibits collecting duct Na⁺ transport. Because agonists are virtually always present *in vivo*, and as confirmed using knockout of EP receptors, the primary physiologic effect of PGE₂ on the collecting duct is natriuretic. Activation of EP1 and EP3 receptors in the collecting duct reduces adenylyl cyclase-dependent cAMP production. Finally, PGE₂ inhibits collecting duct Na⁺ reabsorption through a calcium-dependent mechanism. Taken together, these findings suggest that nonsteroidal anti-inflammatory drug-induced Na⁺ retention is caused, at least in part, by inhibition of collecting duct PGE₂.

Although species differences may exist with regard to the specific EET stereoisomer involved, EETs have been repeatedly shown to inhibit ENaC activity (48–51). The physiologic relevance of EETs in the control of collecting duct Na⁺ transport is largely unknown; however, it is likely that EETs interact with other signaling systems, including mediating

adenosine inhibition of ENaC (52) and stimulating PGE₂ formation (50). Finally, cortical collecting duct EET formation is stimulated by elevated tubule fluid flow, potentially promoting Na⁺ excretion.

Tubule Fluid Flow. Increased collecting duct tubule fluid flow occurs in natriuretic states. The increase in collecting duct tubule fluid flow, at least in the setting of salt loading, results from increased GFR and reduced solute reabsorption by nephron segments proximal to the collecting duct. ENaC is mechanosensitive and is activated by shear stress [reviewed by Kashlan and Kleyman (53)]. Such flow-stimulated ENaC activity would be counterproductive during salt loading; hence, several mechanisms appear to exist to inhibit flow-stimulated ENaC activity. Increases in intracellular or extracellular Na⁺ concentration inhibit ENaC activity. In addition, luminal collecting duct shear stress stimulates a variety of factors that can reduce Na⁺ transport, including 11-EET and 12-EET (54), ATP (55), nitric oxide (NO) (56), PGE₂ (47), and ET-1 (57).

Kinins. The connecting segment and cortical collecting duct are the major sites of renal tissue kallikrein (TK) expression [reviewed by Eladari *et al.* (58)]. TK cleaves kininogen to yield lysyl-BK, which is cleaved by N-aminopeptidase to form BK. BK released by the collecting duct has the potential to interact with BK type 2 (BK2) receptors expressed by the collecting duct. BK activation of BK2 receptors inhibits ENaC activity (59). TK *per se* may regulate ENaC independent of BK (58). The addition of TK to the lumen side of the cortical collecting duct increases ENaC activity associated with increased cleavage of ENaCγ (*i.e.*, removal of the autoinhibitory peptide within ENaCγ). Finally, the BK system should be considered in the context of angiotensin-converting enzyme inhibitors (ACEIs), which are well known to inhibit BK degradation, thereby leading to increased BK levels. This elevation of BK could, in principle, enhance the effect of ACEIs to inhibit ENaC, over and above what angiotensin receptor blockers, which do not inhibit BK degradation, can do. However, the clinical relevance of this difference between ACEIs and angiotensin receptor blockers remains controversial.

NO. Collecting duct NO production is stimulated by tubule fluid flow, ATP, and ET-1 [reviewed by Hyndman and Pollock (60)]. NO reduces ENaC activity (61,62), possibly *via* reduction of AVP-stimulated cAMP accumulation [reviewed by Ortiz and Garvin (62)]. In addition, NO may mediate ATP inhibition of collecting duct ROMK activity (63). Collecting duct NO is likely of physiologic significance because collecting duct-specific knockout of NO synthase 1 causes salt-sensitive hypertension (64).

Peroxisome Proliferator-Activated Receptors. Activation of the peroxisome proliferator-activated receptor-γ by thiazolidinediones is well known to cause Na⁺ retention. Mice with principal cell-specific knockout of peroxisome proliferator-activated receptor-γ are resistant to thiazolidinedione-induced Na⁺ retention (65,66). In addition, other noncollecting duct mechanisms may be involved (67).

Adenosine. Adenosine is derived from ATP and cAMP metabolism. Salt loading increases renal interstitial adenosine; relatively large amounts of adenosine are found in the inner medulla [reviewed by Rieg and Vallon (68)]. Activation of adenosine A₁ receptors inhibits AVP-dependent cAMP-stimulated ENaC activity in the collecting duct.

ATP. Collecting duct cells release ATP into the lumen in response to tubule flow. In addition, dietary salt increases urinary ATP and its metabolites [reviewed by Vallon and Rieg (55)]. The collecting duct lumen expresses low levels of ectonucleotidases, facilitating ATP regulation of collecting duct function. The collecting duct expresses luminal P2Y₂ purinergic receptors, the activation of which inhibits ENaC (55,69,70). P2Y₂ regulation of ENaC is physiologically relevant in that P2Y₂ knockout prevents high-Na diet-induced decreases in ENaC activity. Finally, ATP activation of P2Y₂ may inhibit collecting duct ROMK activity (63).

ET. The collecting duct is the major source of ET-1 in the kidney and may produce more ET-1 than any other cell type in the body [reviewed by Kohan *et al.* (71)]. ET-1 production by the collecting duct is stimulated by luminal shear stress, luminal Na⁺ delivery, and other factors (57,71). In general, collecting duct ET-1 synthesis is increased by extracellular fluid volume expansion. Collecting duct-derived ET-1 can act in an autocrine manner on basolateral ET receptors to regulate Na⁺ transport. The collecting duct expresses relatively high levels of ETB receptors and low levels of ETA receptors. Activation of collecting duct ETB receptors inhibits Na⁺ transport in the cortical collecting duct; this effect is mediated by several signaling systems, including NO (71,72). The physiologic significance of the collecting duct ET system was demonstrated in studies with principal cell-specific knockout of ET-1 or ET receptors. The absence of principal cell ET-1 or both ET receptors causes marked salt-sensitive hypertension (73,74). These findings may be clinically relevant in that ET receptor antagonists, which are currently approved for treatment of pulmonary hypertension and are being studied for the treatment of diabetic nephropathy, can cause significant fluid retention (75). It is possible that this is mediated, at least in part, by blockade of collecting duct ET receptors.

Renin. Renin is synthesized and secreted into the tubule lumen by collecting duct cells [reviewed by Navar *et al.* (76)]. Luminal renin may bind to prorenin receptors on intercalated cells, enhancing its catalytic activity and modulating intercalated cell function. Because angiotensinogen and angiotensin-converting enzymes are present in the distal nephron lumen, secreted renin may stimulate luminal AngII formation leading to enhanced ENaC activity. Because collecting duct renin production is markedly increased by circulating AngII and in the setting of diabetes mellitus, this distal nephron renin system may be of pathophysiologic significance.

Na-K-ATPase

Ultimately, the primary driving force for principal cell Na⁺ reabsorption and K⁺ secretion is provided by the Na⁺-K⁺-ATPase. As in other nephron segments and, indeed, most epithelia, Na⁺-K⁺-ATPase is targeted exclusively to the basolateral membrane of principal cells. Its activity and membrane density are regulated by hormones, such as aldosterone, AVP, and insulin, as well as nonhormonal factors, such as intracellular [Na⁺] and tonicity (77). However, in the control of Na⁺ balance, most notably by aldosterone, the Na⁺-K⁺-ATPase is not the major target of regulation. As addressed below, the bulk

of evidence supports the conclusion that ENaC is the primary site of regulation. This is an important detail for clinicians to be aware of, because it is relevant to commonalities among hypertensive diseases, such as Liddle syndrome, apparent mineralocorticoid excess, and primary aldosteronism, as well as to the treatment of salt-sensitive essential hypertension.

ROMK

A predominant role of the RAAS is to regulate ENaC (18); however, other principal cell transporters, such as ROMK, are also regulated by aldosterone (acting through the MR) (78). ROMK is a critical, but not the sole, mediator of K^+ transport in principal cells (Figure 1). Like ENaC, it is a cation channel. ROMK is highly selective for K^+ over Na^+ , and is inhibited by Ba^{2+} (79). Some of the effects of the RAAS on ROMK may be the result of regulation of with no lysine kinases (WNKs) (2) that, in addition to inhibiting NKCC2 and NCC in the thick ascending limb and distal convoluted tubule, respectively, also inhibit K channels in principal cells (80). Interconnections between RAAS and WNK signaling have been identified, both through effects of AngII and effects of aldosterone, at least in part, mediated by SGK1 (78,81). Assembly and trafficking of ROMK to the cell surface is dictated by a multiprotein complex facilitated by PDZ interactions with scaffold proteins NHERF-1 and NHERF-2 (82). Increased dietary potassium causes a large increase in apical expression of ROMK in the distal convoluted tubule-2, connecting tubule, and collecting duct but not in distal convoluted tubule-1, indicating that multiple regulatory mechanisms are involved in dietary K-regulated ROMK channel function in the distal nephron (83). Recent studies suggest that Cullin3-RING (really interesting new gene) ligases that contain Kelch-like 3 (components of an E_3 ubiquitin ligase complex) target ubiquitinylation of WNK4 and thereby regulate WNK4 levels, which, in turn, regulate levels of ROMK (84). Interestingly, ROMK variants with decreased channel activity have been associated with resistance to hypertension, suggesting that ROMK may also be a determinant of BP control in the general population (85). Hypertension resistance variants of ROMK were found to have decreased channel function as a result of increased sensitivity to the inhibitory effects of a G protein-coupled receptor, which stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis (86).

Cl[−] Transport

There are three contributory pathways for Cl^- transport. First, in principal cells, Cl^- can be reabsorbed or secreted (depending on electrochemical gradient) *via* the cystic fibrosis transmembrane regulator. Cl^- basolateral transport is through a Cl^- channel of the CIC family. Second, Cl^- also moves through a paracellular pathway, at least in part *via* tight junction claudins (2). Finally, Cl^- is transported through intercalated cells (3). Fascinating new data support the idea that, in intercalated cells, the ability of the receptor for aldosterone (the MR) to respond to aldosterone is controlled by phosphorylation (87). This effect influences the ability of these cells to increase Cl^- transport in response to aldosterone, and allows the collecting duct to shift from mediating primarily Na^+-K^+ exchange to mediating both Na^+-K^+ exchange and Na^+-Cl^+ cotransport.

Control of Water Transport in Principal Cells

Overview of Collecting Duct Water Transport

The primary mechanism by which water is reabsorbed in the principal cell is through AVP stimulation of AQP2 expression and accumulation in the luminal plasma membrane (Figure 4). AVP binding to its type 2 receptor (V2R) in the basolateral membrane of principal cells induces a cAMP signaling cascade, which leads rapidly to translocation of AQP2 from intracellular vesicles to the apical membrane. Tubule water enters the cell through AQP2, traverses the cytosol, and exits to the interstitium *via* AQP3 and AQP4, with water movement ultimately being driven by the tubule lumen–interstitium osmotic gradient (88,89). Inactivating mutations in V2R or AQP2 genes lead to nephrogenic diabetes insipidus, a disorder characterized by polyuria and, consequently, polydipsia (90,91) [reviewed by Robben *et al.* (92) and Loonen *et al.* (93)]. Overstimulation of water retention is found in the syndrome of inappropriate release of the antidiuretic hormone and in activating mutations of the V2R, which both lead to hyponatremia with normovolemia.

Basic Biology of AQP2

The principal cell mechanisms for regulation of water reabsorption primarily involve modulation of AQP2 abundance in the luminal plasma membrane. Basolateral AQP3 and, to a lesser extent, AQP4 are necessary for transcellular water movement in the principal cell (94,95), but these channels are mainly constitutively expressed and, therefore, serve a permissive function. By contrast, AQP2 is highly regulated in a complex manner. This involves short-term modulation through alterations in trafficking and long-term regulation through changes in protein expression.

AQP2 has at least five phosphorylation sites that appear to regulate apical membrane insertion and endocytosis [reviewed by Fenton *et al.* (96) and Nedvetsky *et al.* (97)]. PKA is the canonical cAMP-dependent mechanism for AQP2 phosphorylation; however, protein kinase G, protein kinase C, casein kinases, and other kinases may also phosphorylate AQP2 (98). In addition, several phosphatases are likely involved in the regulation of AQP2 phosphorylation and activity. AQP2 phosphorylation near the carboxy terminus at serines S256 and S269 appears to be of primary importance in trafficking to the plasma membrane. Conventionally, PKA-dependent phosphorylation at S256 was considered a priming event for S269 phosphorylation and plasma membrane insertion (99–101); however, recent studies suggest that S256 phosphorylation may be involved in trafficking of AQP2 through the endoplasmic reticulum and Golgi apparatus. Phosphorylation at S269 is important for increasing AQP2 activity by enhancing exocytosis and reducing endocytosis. AQP2 accumulates in clathrin-coated pits and is internalized in a dynamin-dependent manner; this process may be regulated by the phosphorylation state of S256 and S269. Finally, AQP2 is highly phosphorylated at S261 without AVP, but this is reduced by AVP (102,103), suggesting that S261 may also be involved in AQP2 trafficking.

AQP2 internalization is mediated, at least in part, by an unknown ubiquitin ligase, which mediates ubiquitinylation at lysine K270 (104). K270 is essential in this process, because endocytosis of AQP2-K270R, which cannot be further

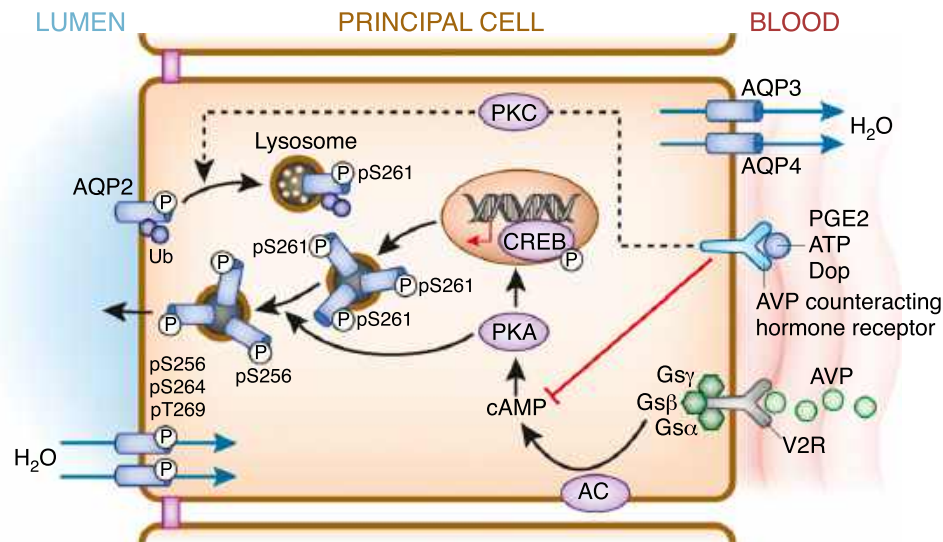


Figure 4. | Hormonal regulation of AQP2 in the principal cell. In states of hypernatremia or hypovolemia, AVP is released from the pituitary and binds its V2R. AVP-bound V2R results in dissociation of the trimeric G protein into a GTP-bound α subunit ($G_{s\alpha}$) and $\beta\gamma$ subunits ($G_{s\beta}$ and $G_{s\gamma}$) of which the first activates AC to generate cAMP. Activation of PKA by cAMP increases AQP2 transcription through phosphorylated activation of the CREB transcription factor and induces translocation of AQP2 from intracellular vesicles to the apical membrane by dephosphorylating AQP2 at Ser261 (pS261) and by phosphorylating AQP2 at Ser256 (pS256), Ser264 (pS264), and Thr269 (pT269). Driven by the transcellular osmotic gradient, prourinary water will then enter the principal cell through AQP2 and exit the cell via AQP3 and AQP4 located in the basolateral membrane of these cells, thereby concentrating urine. Activation of receptors by hormones, such as PGE₂, ATP, and dopamine, counteracts this action of AVP by inducing short-chain ubiquitination of AQP2 at Lys270 (K270), leading to its internalization and lysosomal targeting and degradation. AQP2 pS261 is reinstalled during this internalization process, in which PKC is a central kinase. AC, adenylyl cyclase; AVP, arginine vasopressin; CREB, cAMP-responsive element-binding protein; Dop, dopamine; PKA, protein kinase A; PKC, protein kinase C; Ub, ubiquitin; V2R, vasopressin receptor type 2.

ubiquitinated, was strongly retarded (104). Relevant to this, AQP2 K63-linked ubiquitination is short lived and internalized AQP2 may recycle to the plasma membrane multiple times (105). This suggests that, as with many other membrane proteins, AQP2 is likely deubiquitinated before being targeted for lysosomal degradation. Although the involved ubiquitin ligase and deubiquitinating enzyme are not known, initial studies have identified candidates for further research (106). It is unlikely that this ubiquitin ligase is Nedd4-2; however, the parallels with ENaC regulation are striking.

Another important mechanism for regulation of principal cell water transport is through control of total cell AQP2 levels (*i.e.*, long-term regulation of AQP2 expression) [see the excellent review by Radin *et al.* (107)]. The time frame for maximal changes in AQP2 abundance is not certain, but likely takes several days for up to 10-fold alterations to occur. Changes in principal cell AQP2 degradation or elimination (through exosomes excreted in the urine) do not account for this wide fluctuation in AQP2 abundance. Rather, it appears that transcriptional modulation of AQP2 promoter activity is important. The AQP2 promoter contains cAMP response elements as well as a number of other transcription factor consensus recognition sites.

Stimulators of AQP2

AVP. AVP is the major regulator of AQP2 trafficking and expression in principal cells. AVP exerts multiple effects on cell signaling of the principal cell, foremost of which is stimulation of adenylyl cyclase-dependent cAMP

accumulation and activation of PKA. Another cAMP-activated mediator, Epac, was recently implicated in mediating AQP2 phosphorylation (96,97). AVP increases intracellular $[Ca^{2+}]$ as well as activates Akt (protein kinase B) via the phosphatidylinositol 3-kinase and inhibition of extracellular signal-regulated kinases 1 and 2; some of these processes are likely cAMP dependent. The net effect is that AVP can modulate phosphorylation AQP2 through a variety of protein kinases and likely also through regulation of phosphatases. AVP also reduces AQP2 internalization through decreasing ubiquitination as well as depolymerization of the actin cytoskeleton. AVP increases AQP2 protein expression primarily through enhanced AQP2 gene transcription and, to a lesser extent, through reduced AQP2 degradation.

Interstitial Osmolality. Interstitial osmolality *per se* may regulate AQP2 abundance. AVP-deficient Brattleboro rats start concentrating their urine when water deprived, or when made hypertonic, which all lead to the generation of a hypertonic interstitium (108,109). The tonicity-responsive enhancer-binding protein (TonEBP) may be involved in the hypertonicity response, because its expression is upregulated with hypertonicity (110), inactivating mutations in the tonicity-responsive element in the AQP2 promoter reduced AQP2 expression in mpkCD cells (111), and TonEBP knockout mice or mice transgenic for dominant-negative TonEBP showed decreased AQP2 abundance (112,113).

Other Stimulators. AngII, via angiotensin II type 1 receptors, and aldosterone have been shown to increase AQP2 abundance in mpkCCD cells (114,115), suggesting that the RAAS can control principal cell water reabsorption.

In agreement with this, recent studies found that mice with collecting duct–specific angiotensin II type 1 receptor knockout had reduced urinary concentrating ability associated with reduced AVP-stimulated cAMP accumulation (116). ANP was also suggested to increase principal cell water reabsorption because ANP increased AQP2 phosphorylation at S256 and its translocation to the plasma membrane in inner medullary collecting duct cells (117,118). This, however, is controversial, because others showed that ANP antagonizes AVP-mediated water permeability by decreasing AQP2 phosphorylation and enhancing AQP2 retrieval from the apical membrane (119).

Autocrine and Paracrine Inhibitors of AQP2

Similar to regulation of principal cell Na^+ transport, several autocrine and paracrine factors modulate principal cell water reabsorption. The previous section on Na^+ transport provides details regarding the cell sources and regulation of expression of these factors. In the following, we specifically address these factors in the context of modulation of principal cell water transport.

PGs and Cytochrome P450 Metabolites. As for Na^+ reabsorption in the collecting duct, PGE_2 may increase cAMP-dependent water reabsorption *via* binding to EP4 receptors. In the presence of agonists, PGE_2 inhibits collecting duct water transport through stimulation of EP1 and EP3 receptors leading to inhibition of cAMP production, induction of AQP2 retrieval from the plasma membrane, and Rho-dependent actin depolymerization with resultant inhibition of AQP2 translocation to the plasma membrane (45,46).

Kinins. BK released by the collecting duct can bind to collecting duct BK2 receptors and inhibit AQP2 trafficking to the plasma membrane (120). Whether this is physiologically relevant remains to be determined.

NO. The effects of NO/cGMP on principal cell water transport are controversial, with results varying depending upon the model and conditions studied. NO has been reported by some, but not all, to inhibit collecting duct water transport, the latter through reduction of AVP-stimulated cAMP accumulation [reviewed by Ortiz and Garvin (62)]. By contrast, NO/cAMP has been described to increase AQP2 plasma membrane expression, particularly in *in vitro* studies (98).

Adenosine. Adenosine activation of A_1 receptors inhibits AVP-dependent cAMP-stimulated water reabsorption in the collecting duct [reviewed by Rieg and Vallon (68)]. Because caffeine is shown to exert a diuretic effect through antagonism of the adenosine A_1 receptor, this effect must be the result of nonprincipal cell actions.

ATP. Water loading may increase collecting duct ATP release by increasing cell swelling caused by decreased extracellular tonicity (55). Activation of apical collecting duct P2Y2 receptors inhibits water reabsorption by reducing AVP-stimulated cAMP and increasing PGE_2 production.

ET. ET-1 activation of ETB receptors inhibits AVP-stimulated cAMP accumulation and water reabsorption by the collecting duct. Mice with principal cell–specific knockout of ET-1 demonstrate enhanced water retention and increased cAMP accumulation in response to AVP (121).

Because the regulated transport of ions and water is of vital importance to the role that this tubule cell plays in kidney function and homeostasis, we have addressed the basic transport functions of the principal cell primarily from the standpoint of regulation. The central role of two different hormone-transporter pairs was emphasized: aldosterone and ENaC for control of ion transport, and AVP and AQP2 for control of water transport. However, the complex regulation of principal cell function is underscored by the panoply of hormonal, autocrine, paracrine, and physical factors that regulate its activities. A few examples are as follows: ATP, PGE_2 , and ET, which inhibit both Na^+ and water transport; AngII, which stimulates both Na^+ and water transport; and insulin, which selectively stimulates Na^+ transport. Under normal physiologic conditions, these diverse regulators exert their effects in various combinations to provide context-appropriate integrated responses to different environmental conditions.

Understanding the signaling and transport mechanisms that underlie principal cell function is valuable to practicing clinicians, both because it enhances our appreciation of the kidney and its role in homeostasis, and because it provides a foundation for greater depth and flexibility in our approach to diagnosis and treatment of the wide array of fluid and electrolyte disorders we encounter.

Disclosures

None.

References

- Palmer LG, Frindt G: Na^+ and K^+ transport by the renal connecting tubule. *Curr Opin Nephrol Hypertens* 16: 477–483, 2007
- Hou J, Rajagopal M, Yu AS: Claudins and the kidney. *Annu Rev Physiol* 75: 479–501, 2013
- Schuster VL, Stokes JB: Chloride transport by the cortical and outer medullary collecting duct. *Am J Physiol* 253: F203–F212, 1987
- Palmer LG, Patel A, Frindt G: Regulation and dysregulation of epithelial Na^+ channels. *Clin Exp Nephrol* 16: 35–43, 2012
- Wang WH, Giebisch G: Regulation of potassium (K) handling in the renal collecting duct. *Pflügers Arch* 458: 157–168, 2009
- Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC: Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* 367: 463–467, 1994
- Jasti J, Furukawa H, Gonzales EB, Gouaux E: Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature* 449: 316–323, 2007
- Kashlan OB, Kleyman TR: ENaC structure and function in the wake of a resolved structure of a family member. *Am J Physiol Renal Physiol* 301: F684–F696, 2011
- Soundararajan R, Melters D, Shih IC, Wang J, Pearce D: Epithelial sodium channel regulated by differential composition of a signaling complex. *Proc Natl Acad Sci U S A* 106: 7804–7809, 2009
- Soundararajan R, Ziera T, Koo E, Ling K, Wang J, Borden SA, Pearce D: Scaffold protein connector enhancer of kinase suppressor of Ras isoform 3 (CNK3) coordinates assembly of a multiprotein epithelial sodium channel (ENaC)-regulatory complex. *J Biol Chem* 287: 33014–33025, 2012
- Yoo D, Flagg TP, Olsen O, Raghuram V, Foscett JK, Welling PA: Assembly and trafficking of a multiprotein ROMK (Kir 1.1) channel complex by PDZ interactions. *J Biol Chem* 279: 6863–6873, 2004
- Hughey RP, Bruns JB, Kinlough CL, Harkleroad KL, Tong Q, Carattino MD, Johnson JP, Stockand JD, Kleyman TR: Epithelial sodium channels are activated by furin-dependent proteolysis. *J Biol Chem* 279: 18111–18114, 2004

Summary

The principal cell is arguably the most highly regulated cell type in the kidney tubules, if not in all mammalian epithelia.

13. Passero CJ, Mueller GM, Rondon-Berrios H, Tofovic SP, Hughey RP, Kleyman TR: Plasmin activates epithelial Na⁺ channels by cleaving the gamma subunit. *J Biol Chem* 283: 36586–36591, 2008
14. Svenningsen P, Bistrup C, Friis UG, Bertog M, Haerteis S, Krueger B, Stubbe J, Jensen ON, Thiesson HC, Uhrenholt TR, Jespersen B, Jensen BL, Korbmacher C, Skott O: Plasmin in nephrotic urine activates the epithelial sodium channel. *J Am Soc Nephrol* 20: 299–310, 2009
15. Warnock DG, Rossier BC: Renal sodium handling: The role of the epithelial sodium channel. *J Am Soc Nephrol* 16: 3151–3153, 2005
16. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR Jr, Ulick S, Milora RV, Findling JW, Canessa CM, Rossier BC, Lifton RP: Liddle's syndrome: Heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* 79: 407–414, 1994
17. Soundararajan R, Pearce D, Hughey RP, Kleyman TR: Role of epithelial sodium channels and their regulators in hypertension. *J Biol Chem* 285: 30363–30369, 2010
18. Pearce D, Bhalla V, Funder JW, Stokes JB: Aldosterone regulation of ion transport. In: *The Kidney*, edited by Brenner BM, Philadelphia, Saunders Elsevier, 2012
19. Frindt G, Ergonul Z, Palmer LG: Surface expression of epithelial Na channel protein in rat kidney. *J Gen Physiol* 131: 617–627, 2008
20. Verrey F, Schaerer E, Zoerkler P, Paccolat MP, Geering K, Kraehenbuhl JP, Rossier BC: Regulation by aldosterone of Na⁺, K⁺-ATPase mRNAs, protein synthesis, and sodium transport in cultured kidney cells. *J Cell Biol* 104: 1231–1237, 1987
21. Soundararajan R, Wang J, Melters D, Pearce D: Glucocorticoid-induced Leucine zipper 1 stimulates the epithelial sodium channel by regulating serum- and glucocorticoid-induced kinase 1 stability and subcellular localization. *J Biol Chem* 285: 39905–39913, 2010
22. Lu M, Wang J, Jones KT, Ives HE, Feldman ME, Yao LJ, Shokat KM, Ashrafi K, Pearce D: mTOR complex-2 activates ENaC by phosphorylating SGK1. *J Am Soc Nephrol* 21: 811–818, 2010
23. McCormick JA, Bhalla V, Pao AC, Pearce D: SGK1: A rapid aldosterone-induced regulator of renal sodium reabsorption. *Physiology (Bethesda)* 20: 134–139, 2005
24. Debonneville C, Flores SY, Kamynina E, Plant PJ, Tauxe C, Thomas MA, Münster C, Chraïbi A, Pratt JH, Horisberger JD, Pearce D, Loffing J, Staub O: Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. *EMBO J* 20: 7052–7059, 2001
25. Kabra R, Knight KK, Zhou R, Snyder PM: Nedd4-2 induces endocytosis and degradation of proteolytically cleaved epithelial Na⁺ channels. *J Biol Chem* 283: 6033–6039, 2008
26. Bhalla V, Soundararajan R, Pao AC, Li H, Pearce D: Dis-inhibitory pathways for control of sodium transport: Regulation of ENaC by SGK1 and GILZ. *Am J Physiol Renal Physiol* 291: F714–F721, 2006
27. Zhang W, Xia X, Reisenauer MR, Rieg T, Lang F, Kuhl D, Vallon V, Kone BC: Aldosterone-induced Sgk1 relieves Dot1a-Af9-mediated transcriptional repression of epithelial Na⁺ channel alpha. *J Clin Invest* 117: 773–783, 2007
28. Ronzaud C, Loffing-Cueni D, Hausel P, Debonneville A, Malsure SR, Fowler-Jaeger N, Boase NA, Perrier E, Maillard M, Yang B, Stokes JB, Koesters R, Kumar S, Hummler E, Loffing J, Staub O: Renal tubular NEDD4-2 deficiency causes NCC-mediated salt-dependent hypertension. *J Clin Invest* 123: 657–665, 2013
29. Lang F, Huang DY, Vallon V: SGK, renal function and hypertension. *J Nephrol* 23[Suppl 16]: S124–S129, 2010
30. Ackermann TF, Boini KM, Beier N, Scholz W, Fuchss T, Lang F: EMD638683, a novel SGK inhibitor with antihypertensive potency. *Cell Physiol Biochem* 28: 137–146, 2011
31. van der Lubbe N, Zietse R, Hoorn EJ: Effects of angiotensin II on kinase-mediated sodium and potassium transport in the distal nephron. *Curr Opin Nephrol Hypertens* 22: 120–126, 2013
32. Tremblay J, Gerzer R, Vinay P, Pang SC, Béliveau R, Hamet P: The increase of cGMP by atrial natriuretic factor correlates with the distribution of particulate guanylate cyclase. *FEBS Lett* 181: 17–22, 1985
33. Hayek S, Nemer M: Cardiac natriuretic peptides: From basic discovery to clinical practice. *Cardiovasc Ther* 29: 362–376, 2011
34. Guo LJ, Alli AA, Eaton DC, Bao HF: ENaC is regulated by natriuretic peptide receptor-dependent cGMP signaling. *Am J Physiol Renal Physiol* 304: F930–F937, 2013
35. Gambaryan S, Wagner C, Smolenski A, Walter U, Poller W, Haase W, Kurtz A, Lohmann SM: Endogenous or overexpressed cGMP-dependent protein kinases inhibit cAMP-dependent renin release from rat isolated perfused kidney, microdissected glomeruli, and isolated juxtaglomerular cells. *Proc Natl Acad Sci U S A* 95: 9003–9008, 1998
36. Kudo T, Baird A: Inhibition of aldosterone production in the adrenal glomerulosa by atrial natriuretic factor. *Nature* 312: 756–757, 1984
37. Rocchini AP: Obesity hypertension, salt sensitivity and insulin resistance. *Nutr Metab Cardiovasc Dis* 10: 287–294, 2000
38. Baum M: Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *J Clin Invest* 79: 1104–1109, 1987
39. Reaven GM: The kidney: An unwilling accomplice in syndrome X. *Am J Kidney Dis* 30: 928–931, 1997
40. Muniyappa R, Quon MJ: Insulin action and insulin resistance in vascular endothelium. *Curr Opin Clin Nutr Metab Care* 10: 523–530, 2007
41. Snyder PM, Olson DR, Kabra R, Zhou R, Steines JC: cAMP and serum and glucocorticoid-inducible kinase (SGK) regulate the epithelial Na(+) channel through convergent phosphorylation of Nedd4-2. *J Biol Chem* 279: 45753–45758, 2004
42. Bankir L, Bichet DG, Bouby N: Vasopressin V2 receptors, ENaC, and sodium reabsorption: A risk factor for hypertension? *Am J Physiol Renal Physiol* 299: F917–F928, 2010
43. Loesch A, Unwin R, Gandhi V, Burnstock G: Sympathetic nerve varicosities in close apposition to basolateral membranes of collecting duct epithelial cells of rat kidney. *Nephron, Physiol* 113: 15–21, 2009
44. Wallace DP, Reif G, Hedge AM, Thrasher JB, Pietrow P: Adrenergic regulation of salt and fluid secretion in human medullary collecting duct cells. *Am J Physiol Renal Physiol* 287: F639–F648, 2004
45. Breyer MD, Breyer RM: Prostaglandin E receptors and the kidney. *Am J Physiol Renal Physiol* 279: F12–F23, 2000
46. Boone M, Deen PM: Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. *Pflügers Arch* 456: 1005–1024, 2008
47. Flores D, Liu Y, Liu W, Satlin LM, Rohatgi R: Flow-induced prostaglandin E2 release regulates Na and K transport in the collecting duct. *Am J Physiol Renal Physiol* 303: F632–F638, 2012
48. Capdevila J, Wang W: Role of cytochrome P450 epoxygenase in regulating renal membrane transport and hypertension. *Curr Opin Nephrol Hypertens* 22: 163–169, 2013
49. Pavlov TS, Ilatovskaya DV, Levchenko V, Mattson DL, Roman RJ, Staruschenko A: Effects of cytochrome P-450 metabolites of arachidonic acid on the epithelial sodium channel (ENaC). *Am J Physiol Renal Physiol* 301: F672–F681, 2011
50. Sakairi Y, Jacobson HR, Noland TD, Capdevila JH, Falck JR, Breyer MD: 5,6-EET inhibits ion transport in collecting duct by stimulating endogenous prostaglandin synthesis. *Am J Physiol* 268: F931–F939, 1995
51. Wei Y, Lin DH, Kemp R, Yaddanapudi GS, Nasjletti A, Falck JR, Wang WH: Arachidonic acid inhibits epithelial Na channel via cytochrome P450 (CYP) epoxygenase-dependent metabolic pathways. *J Gen Physiol* 124: 719–727, 2004
52. Wei Y, Sun P, Wang Z, Yang B, Carroll MA, Wang WH: Adenosine inhibits ENaC via cytochrome P-450 epoxygenase-dependent metabolites of arachidonic acid. *Am J Physiol Renal Physiol* 290: F1163–F1168, 2006
53. Kashlan OB, Kleyman TR: Epithelial Na(+) channel regulation by cytoplasmic and extracellular factors. *Exp Cell Res* 318: 1011–1019, 2012
54. Sun P, Liu W, Lin DH, Yue P, Kemp R, Satlin LM, Wang WH: Epoxyeicosatrienoic acid activates BK channels in the cortical collecting duct. *J Am Soc Nephrol* 20: 513–523, 2009

55. Vallon V, Rieg T: Regulation of renal NaCl and water transport by the ATP/UTP/P2Y2 receptor system. *Am J Physiol Renal Physiol* 301: F463–F475, 2011
56. Cai Z, Xin J, Pollock DM, Pollock JS: Shear stress-mediated NO production in inner medullary collecting duct cells. *Am J Physiol Renal Physiol* 279: F270–F274, 2000
57. Lyon-Roberts B, Strait KA, van Peursem E, Kittikulsuth W, Pollock JS, Pollock DM, Kohan DE: Flow regulation of collecting duct endothelin-1 production. *Am J Physiol Renal Physiol* 300: F650–F656, 2011
58. Eladari D, Chambrey R, Peti-Peterdi J: A new look at electrolyte transport in the distal tubule. *Annu Rev Physiol* 74: 325–349, 2012
59. Zaika O, Mamenko M, O'Neil RG, Pochynyuk O: Bradykinin acutely inhibits activity of the epithelial Na⁺ channel in mammalian aldosterone-sensitive distal nephron. *Am J Physiol Renal Physiol* 300: F1105–F1115, 2011
60. Hyndman KA, Pollock JS: Nitric oxide and the A and B of endothelin of sodium homeostasis. *Curr Opin Nephrol Hypertens* 22: 26–31, 2013
61. Helms MN, Yu L, Malik B, Kleinhenz DJ, Hart CM, Eaton DC: Role of SGK1 in nitric oxide inhibition of ENaC in Na⁺-transporting epithelia. *Am J Physiol Cell Physiol* 289: C717–C726, 2005
62. Ortiz PA, Garvin JL: Role of nitric oxide in the regulation of nephron transport. *Am J Physiol Renal Physiol* 282: F777–F784, 2002
63. Lu M, MacGregor GG, Wang W, Giebisch G: Extracellular ATP inhibits the small-conductance K channel on the apical membrane of the cortical collecting duct from mouse kidney. *J Gen Physiol* 116: 299–310, 2000
64. Hyndman KA, Boesen EI, Elmarakby AA, Brands MW, Huang P, Kohan DE, Pollock DM, Pollock JS: Renal collecting duct NOS1 maintains fluid-electrolyte homeostasis and blood pressure. *Hypertension* 62: 91–98, 2013
65. Guan Y, Hao C, Cha DR, Rao R, Lu W, Kohan DE, Magnuson MA, Redha R, Zhang Y, Breyer MD: Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption. *Nat Med* 11: 861–866, 2005
66. Zhang H, Zhang A, Kohan DE, Nelson RD, Gonzalez FJ, Yang T: Collecting duct-specific deletion of peroxisome proliferator-activated receptor gamma blocks thiazolidinedione-induced fluid retention. *Proc Natl Acad Sci U S A* 102: 9406–9411, 2005
67. Pavlov TS, Imig JD, Staruschenko A: Regulation of ENaC-mediated sodium reabsorption by peroxisome proliferator-activated receptors. *PPAR Res* 2010: 703735, 2010
68. Rieg T, Vallon V: ATP and adenosine in the local regulation of water transport and homeostasis by the kidney. *Am J Physiol Regul Integr Comp Physiol* 296: R419–R427, 2009
69. Pochynyuk O, Bugaj V, Rieg T, Insel PA, Mironova E, Vallon V, Stockand JD: Paracrine regulation of the epithelial Na⁺ channel in the mammalian collecting duct by purinergic P2Y2 receptor tone. *J Biol Chem* 283: 36599–36607, 2008
70. Wildman SS, Marks J, Turner CM, Yew-Booth L, Peppiatt-Wildman CM, King BF, Shirley DG, Wang W, Unwin RJ: Sodium-dependent regulation of renal amiloride-sensitive currents by apical P2 receptors. *J Am Soc Nephrol* 19: 731–742, 2008
71. Kohan DE, Rossi NF, Inscho EW, Pollock DM: Regulation of blood pressure and salt homeostasis by endothelin. *Physiol Rev* 91: 1–77, 2011
72. Bugaj V, Mironova E, Kohan DE, Stockand JD: Collecting duct-specific endothelin B receptor knockout increases ENaC activity. *Am J Physiol Cell Physiol* 302: C188–C194, 2012
73. Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE: Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. *J Clin Invest* 114: 504–511, 2004
74. Ge Y, Bagnall A, Stricklett PK, Webb D, Kotelevtsev Y, Kohan DE: Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. *Am J Physiol Renal Physiol* 295: F1635–F1640, 2008
75. Barton M, Kohan DE: Endothelin antagonists in clinical trials: Lessons learned. *Contrib Nephrol* 172: 255–260, 2011
76. Navar LG, Kobori H, Prieto MC, Gonzalez-Villalobos RA: Intratubular renin-angiotensin system in hypertension. *Hypertension* 57: 355–362, 2011
77. Vinciguerra M, Mordasini D, Vandewalle A, Feraille E: Hormonal and nonhormonal mechanisms of regulation of the Na, K-pump in collecting duct principal cells. *Semin Nephrol* 25: 312–321, 2005
78. McCormick JA, Ellison DH: The WNKs: Atypical protein kinases with pleiotropic actions. *Physiol Rev* 91: 177–219, 2011
79. Ho K, Nichols CG, Lederer WJ, Lytton J, Vassilev PM, Kanazirska MV, Hebert SC: Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature* 362: 31–38, 1993
80. Kahle KT, Wilson FH, Leng Q, Lalioti MD, O'Connell AD, Dong K, Rapson AK, MacGregor GG, Giebisch G, Hebert SC, Lifton RP: WNK4 regulates the balance between renal NaCl reabsorption and K⁺ secretion. *Nat Genet* 35: 372–376, 2003
81. Hoorn EJ, Nelson JH, McCormick JA, Ellison DH: The WNK kinase network regulating sodium, potassium, and blood pressure. *J Am Soc Nephrol* 22: 605–614, 2011
82. Yoo D, Flagg TP, Olsen O, Raghuram V, Foskett JK, Welling PA: Assembly and trafficking of a multiprotein ROMK (Kir 1.1) channel complex by PDZ interactions. *J Biol Chem* 279: 6863–6873, 2004
83. Wade JB, Fang L, Coleman RA, Liu J, Grimm PR, Wang T, Welling PA: Differential regulation of ROMK (Kir1.1) in distal nephron segments by dietary potassium. *Am J Physiol Renal Physiol* 300: F1385–F1393, 2011
84. Shibata S, Zhang J, Puthumana J, Stone KL, Lifton RP: Kelch-like 3 and Cullin 3 regulate electrolyte homeostasis via ubiquitination and degradation of WNK4. *Proc Natl Acad Sci U S A* 110: 7838–7843, 2013
85. Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP: Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet* 40: 592–599, 2008
86. Fang L, Li D, Welling PA: Hypertension resistance polymorphisms in ROMK (Kir1.1) alter channel function by different mechanisms. *Am J Physiol Renal Physiol* 299: F1359–F1364, 2010
87. Shibata S, Rinehart J, Zhang J, Moeckel G, Castañeda-Bueno M, Stiegler AL, Boggon TJ, Gamba G, Lifton RP: Mineralocorticoid receptor phosphorylation regulates ligand binding and renal response to volume depletion and hyperkalemia. *Cell Metab* 18: 660–671, 2013
88. Ishibashi K, Sasaki S, Fushimi K, Yamamoto T, Kuwahara M, Marumo F: Immunolocalization and effect of dehydration on AQP3, a basolateral water channel of kidney collecting ducts. *Am J Physiol* 272: F235–F241, 1997
89. Kim SW, Gresz V, Rojek A, Wang W, Verkman AS, Frøkiaer J, Nielsen S: Decreased expression of AQP2 and AQP4 water channels and Na,K-ATPase in kidney collecting duct in AQP3 null mice. *Biol Cell* 97: 765–778, 2005
90. Deen PMT, Weghuis DO, Sinke RJ, Geurts van Kessel A, Wieringa B, van Os CH: Assignment of the human gene for the water channel of renal collecting duct Aquaporin 2 (AQP2) to chromosome 12 region q12—>q13. *Cytogenet Cell Genet* 66: 260–262, 1994
91. Rosenthal W, Seibold A, Antaramian A, Lonergan M, Arthus-M-F, Hendy GN, Birnbaumer M, Bichet DG: Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature* 359: 233–235, 1992
92. Robben JH, Knoers NV, Deen PM: Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol* 291: F257–F270, 2006
93. Loonen AJ, Knoers NV, van Os CH, Deen PM: Aquaporin 2 mutations in nephrogenic diabetes insipidus. *Semin Nephrol* 28: 252–265, 2008
94. Ma T, Song Y, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS: Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc Natl Acad Sci U S A* 97: 4386–4391, 2000
95. Chou C-L, Ma T, Yang B, Knepper MA, Verkman AS: Fourfold reduction of water permeability in inner medullary collecting

- duct of aquaporin-4 knockout mice. *Am J Physiol* 274: C549–C554, 1998
96. Fenton RA, Pedersen CN, Moeller HB: New insights into regulated aquaporin-2 function. *Curr Opin Nephrol Hypertens* 22: 551–558, 2013
 97. Nedvetsky PI, Tamma G, Beulshausen S, Valenti G, Rosenthal W, Klusmann E: Regulation of aquaporin-2 trafficking. *Handb Exp Pharmacol* 190: 133–157, 2009
 98. Bouley R, Hasler U, Lu HA, Nunes P, Brown D: Bypassing vasopressin receptor signaling pathways in nephrogenic diabetes insipidus. *Semin Nephrol* 28: 266–278, 2008
 99. Christensen BM, Zelenina M, Aperia A, Nielsen S: Localization and regulation of PKA-phosphorylated AQP2 in response to V(2)-receptor agonist/antagonist treatment. *Am J Physiol Renal Physiol* 278: F29–F42, 2000
 100. van Balkom BWM, Savelkoul PJ, Markovich D, Hofman E, Nielsen S, van der Sluijs P, Deen PM: The role of putative phosphorylation sites in the targeting and shuttling of the aquaporin-2 water channel. *J Biol Chem* 277: 41473–41479, 2002
 101. Fushimi K, Sasaki S, Marumo F: Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. *J Biol Chem* 272: 14800–14804, 1997
 102. Hoffert JD, Nielsen J, Yu MJ, Pisitkun T, Schleicher SM, Nielsen S, Knepper MA: Dynamics of aquaporin-2 serine-261 phosphorylation in response to short-term vasopressin treatment in collecting duct. *Am J Physiol Renal Physiol* 292: F691–F700, 2007
 103. Tamma G, Robben JH, Trimpert C, Boone M, Deen PM: Regulation of AQP2 localization by S256 and S261 phosphorylation and ubiquitination. *Am J Physiol Cell Physiol* 300: C636–C646, 2011
 104. Kamsteeg EJ, Hendriks G, Boone M, Konings IB, Oorschot V, van der Sluijs P, Klumperman J, Deen PM: Short-chain ubiquitination mediates the regulated endocytosis of the aquaporin-2 water channel. *Proc Natl Acad Sci U S A* 103: 18344–18349, 2006
 105. Katsura T, Ausiello DA, Brown D: Direct demonstration of aquaporin-2 water channel recycling in stably transfected LLC-PK1 epithelial cells. *Am J Physiol* 270: F548–F553, 1996
 106. Lee YJ, Lee JE, Choi HJ, Lim JS, Jung HJ, Baek MC, Frøkiær J, Nielsen S, Kwon TH: E3 ubiquitin-protein ligases in rat kidney collecting duct: Response to vasopressin stimulation and withdrawal. *Am J Physiol Renal Physiol* 301: F883–F896, 2011
 107. Radin MJ, Yu MJ, Støedkilde L, Miller RL, Hoffert JD, Frøkiær J, Pisitkun T, Knepper MA: Aquaporin-2 regulation in health and disease. *Vet Clin Pathol* 41: 455–470, 2012
 108. Valtin H, Edwards BR: GFR and the concentration of urine in the absence of vasopressin. Berliner-Davidson re-explored. *Kidney Int* 31: 634–640, 1987
 109. Li C, Wang W, Summer SN, Cadnapaphornchai MA, Falk S, Umenishi F, Schrier RW: Hyperosmolality in vivo upregulates aquaporin 2 water channel and Na-K-2Cl co-transporter in Brattleboro rats. *J Am Soc Nephrol* 17: 1657–1664, 2006
 110. Sheen MR, Kim JA, Lim SW, Jung JY, Han KH, Jeon US, Park SH, Kim J, Kwon HM: Interstitial tonicity controls TonEBP expression in the renal medulla. *Kidney Int* 75: 518–525, 2009
 111. Hasler U, Jeon US, Kim JA, Mordasini D, Kwon HM, Féraillé E, Martin PY: Tonicity-responsive enhancer binding protein is an essential regulator of aquaporin-2 expression in renal collecting duct principal cells. *J Am Soc Nephrol* 17: 1521–1531, 2006
 112. Lam AK, Ko BC, Tam S, Morris R, Yang JY, Chung SK, Chung SS: Osmotic response element-binding protein (OREBP) is an essential regulator of the urine concentrating mechanism. *J Biol Chem* 279: 48048–48054, 2004
 113. López-Capapé M, Golmayo L, Lorenzo G, Gallego N, Barrio R: Hypothalamic adipic hypernatraemia syndrome with normal overregulation of vasopressin. *Eur J Pediatr* 163: 580–583, 2004
 114. Li C, Wang W, Rivard CJ, Lanaspá MA, Summer S, Schrier RW: Molecular mechanisms of angiotensin II stimulation on aquaporin-2 expression and trafficking. *Am J Physiol Renal Physiol* 300: F1255–F1261, 2011
 115. Hasler U, Leroy V, Martin PY, Féraillé E: Aquaporin-2 abundance in the renal collecting duct: New insights from cultured cell models. *Am J Physiol Renal Physiol* 297: F10–F18, 2009
 116. Stegbauer J, Gurley SB, Sparks MA, Woznowski M, Kohan DE, Yan M, Lech RW, Coffman TM: AT1 receptors in the collecting duct directly modulate the concentration of urine. *J Am Soc Nephrol* 22: 2237–2246, 2011
 117. Silver MA: The natriuretic peptide system: Kidney and cardiovascular effects. *Curr Opin Nephrol Hypertens* 15: 14–21, 2006
 118. Boone M, Kortenoeven M, Robben JH, Deen PM: Effect of the cGMP pathway on AQP2 expression and translocation: Potential implications for nephrogenic diabetes insipidus. *Nephrol Dial Transplant* 25: 48–54, 2010
 119. Klokke J, Langehanenberg P, Kemper B, Kosmeier S, von Bally G, Riethmüller C, Wunder F, Sindic A, Pavenstädt H, Schlatter E, Edemir B: Atrial natriuretic peptide and nitric oxide signaling antagonizes vasopressin-mediated water permeability in inner medullary collecting duct cells. *Am J Physiol Renal Physiol* 297: F693–F703, 2009
 120. Tamma G, Carmosino M, Svelto M, Valenti G: Bradykinin signaling counteracts cAMP-elicited aquaporin 2 translocation in renal cells. *J Am Soc Nephrol* 16: 2881–2889, 2005
 121. Ge Y, Ahn D, Stricklett PK, Hughes AK, Yanagisawa M, Verbalis JG, Kohan DE: Collecting duct-specific knockout of endothelin-1 alters vasopressin regulation of urine osmolality. *Am J Physiol Renal Physiol* 288: F912–F920, 2005
 122. Kashlan OB, Adelman JL, Okumura S, Blobner BM, Zuzek Z, Hughey RP, Kleyman TR, Grabe M: Constraint-based, homology model of the extracellular domain of the epithelial Na⁺ channel α subunit reveals a mechanism of channel activation by proteases. *J Biol Chem* 286: 649–660, 2011

Published online ahead of print. Publication date available at www.cjasn.org.



Collecting Duct Intercalated Cell Function and Regulation

Ankita Roy,* Mohammad M. Al-bataineh,* and Núria M. Pastor-Soler*[†]

Abstract

Intercalated cells are kidney tubule epithelial cells with important roles in the regulation of acid-base homeostasis. However, in recent years the understanding of the function of the intercalated cell has become greatly enhanced and has shaped a new model for how the distal segments of the kidney tubule integrate salt and water reabsorption, potassium homeostasis, and acid-base status. These cells appear in the late distal convoluted tubule or in the connecting segment, depending on the species. They are most abundant in the collecting duct, where they can be detected all the way from the cortex to the initial part of the inner medulla. Intercalated cells are interspersed among the more numerous segment-specific principal cells. There are three types of intercalated cells, each having distinct structures and expressing different ensembles of transport proteins that translate into very different functions in the processing of the urine. This review includes recent findings on how intercalated cells regulate their intracellular milieu and contribute to acid-base regulation and sodium, chloride, and potassium homeostasis, thus highlighting their potential role as targets for the treatment of hypertension. Their novel regulation by paracrine signals in the collecting duct is also discussed. Finally, this article addresses their role as part of the innate immune system of the kidney tubule.

Clin J Am Soc Nephrol 10: 305–324, 2015. doi: 10.2215/CJN.08880914

Introduction

Intercalated cells are epithelial cells traditionally associated with the regulation of acid-base homeostasis in distal segments of the kidney tubule (Figure 1) (1). These cells also participate in potassium and ammonia transport and have a role in the innate immune system. Many early studies emphasized that these tubule segments were not part of the classic nephron because they arise from the mesonephric kidney or Wolffian duct, which also gives origin to the male excurrent duct (2). Most collecting duct cells express the epithelial sodium channel (ENaC) and are grouped together as principal cells (3,4). Until recently, our understanding of the collecting duct and the roles of intercalated cells has lagged behind that of other segments. The collecting duct was initially described as not having a specialized function or as having a role only in water reabsorption (5,6).

Intercalated cells are essential in the response to acid-base status of the organism, and they help dispose of acid that is generated by dietary intake and cannot be eliminated *via* the lungs, the so-called fixed or nonvolatile acid (Figure 2). The kidney contributes to acid-base homeostasis by recovering filtered bicarbonate in the proximal tubule. Distally, intercalated cells generate new bicarbonate, which is consumed by the titration of nonvolatile acid (7). Dysfunction of the proximal tubule, where approximately 90% of the bicarbonate is reabsorbed, leads to proximal renal tubular acidosis (8). The connecting segment and collecting duct rely mostly on their intercalated cells to reabsorb the normally smaller amount of residual bicarbonate. In addition, intercalated cells participate in the excretion of

ammonia/ammonium, a topic reviewed in a separate article in this series (9).

The relevance of intercalated cell dysfunction in clinical scenarios is often not as evident as the relevance of principal cell dysfunction, such as in patients who present with diabetes insipidus or the syndrome of inappropriate antidiuretic hormone secretion. In clinical practice, intercalated cell dysfunction is most often associated with metabolic acidosis, although histologic or laboratory confirmation of this dysfunction is seldom performed in the general acute care setting. Moreover, the contribution of intercalated cells in preventing acidemia is often eclipsed by the coordinated compensatory roles of the lung, bone, and more proximal kidney tubule segments. Nonetheless, animals subjected to dietary acid loading have significant increases in the luminal (facing the urine) surface area of intercalated cells, changes that begin within a few hours from the change in diet (reviewed in references 7,10).

Until very recently, intercalated cells were not thought to contribute to extracellular fluid volume regulation, yet now they are firmly established as important contributors to collecting duct NaCl transepithelial transport and the protection of intravascular volume in concert with principal cells (Figure 2) (reviewed by Eladari *et al.* [4]). An impressive new study has now established that, *in vivo*, intercalated cells regulate their intracellular volume by a mechanism involving the vacuolar H⁺-ATPase (H⁺-ATPase; also referred to as V-ATPase in the literature). This study is important because it establishes that although most animal cells rely on the Na⁺/K⁺-ATPase (Na,K-ATPase) to energize other

*Renal-Electrolyte Division, Department of Medicine; and
[†]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
A.R. and M.M.A. contributed equally to this work.

Correspondence: Dr. Núria M. Pastor-Soler, Renal-Electrolyte Division, A915 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261. Email: pastorn@pitt.edu

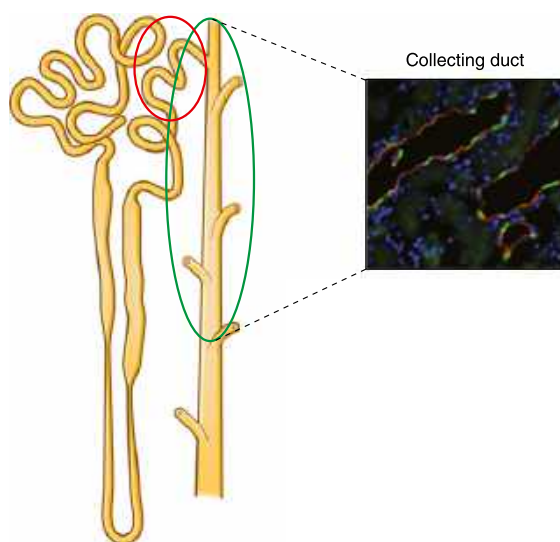


Figure 1. | Intercalated cells in rat collecting duct. The cartoon and confocal micrograph illustrate the intercalated cell distribution along the kidney tubule and within the epithelium. Intercalated cells were detected in the cortical and outer medullary collecting duct (green oval) by immunofluorescence labeling using an antibody against one of the H^+ -ATPase subunits (green). Intercalated cells are also located in the connecting segment (red circle). The apical or luminal labeling of the H^+ -ATPase indicates that these cells are mostly type A intercalated cells (A-IC). Principal cells were labeled with an antibody against the water channel aquaporin-2 (red). Modified from reference 9, with permission.

transport processes, intercalated cells instead rely on the H^+ -ATPase (11). Therefore, now it also must be considered that cell types with high H^+ -ATPase levels may use this pump for more than just acid-base regulation.

Intercalated Cell Distribution, Nomenclature, Morphology, and Developmental Considerations

The cells lining the proximal tubule, thin limb, and thick ascending limb have a homogeneous ultrastructural appearance. Tubule-specific cells function as a unit within each segment. Distal to the macula densa, however, the epithelia of the distal convoluted tubule, connecting segment, and collecting duct are lined by different types of cells. Most cells in these segments are considered segment-specific principal cells, while the other cells are grouped together and called intercalated cells (5,12). An impediment to the study of these cells has been their pleomorphism and their isolation within the epithelium; they sit surrounded by principal cells. Intercalated cells vary in morphology, localization, and abundance, and these differences are evident not only among species but also among individuals from the same species. In addition, few cell lines replicate their phenotypes in culture. Some of the available immortalized intercalated cell models include the rabbit Clone-C cells, the mouse OMCD_{isr}, and the canine MDCKC¹¹ (13–15). The Clone-C cell line is particularly useful because it can switch phenotypes from type B to type A intercalated cells (Figure 2) depending on culture conditions (16,17). Of note, many very relevant studies that have uncovered important regulatory pathways in

intercalated cells have been performed in cell lines of inner medullary origin, such as the mIMCD3 cell line (18,19).

In the collecting duct, principal and intercalated cells form a “salt and pepper” epithelium (Figures 1 and 2) (20). This more primitive appearance of the collecting duct is similar to that of frog skin, reptilian bladder, and fish gills (20–22). In the kidney, the transition from the early part of the distal convoluted tubule to an epithelium containing both principal and intercalated cells coincides with the transition to segments that are derived from the ureteric bud outpouching of the Wolffian duct (2). The ratio of principal to intercalated cells varies slightly between tubular segments, with a ratio of 2:1 in outer medullary collecting duct segments and 3:1 in the cortical collecting duct. The ratio also varies between species (10,23).

Intercalated cells have had obscure names such as “dark cell” and “special cell,” a terminology that reflected how little was known about their function from the time of their discovery in 1876 until the late 1960s (24). Their ultrastructural description, however, has remained quite consistent over the years. Intercalated cells are also called “mitochondria-rich cells,” reflecting the high levels of round mitochondria at their apical pole, or in their cytoplasm, a distribution that was quite different from that of other kidney tubule cells, which accumulated mitochondria around the basolateral membrane (Figure 3) (24–27). These cells also have “numerous irregular apical microvilli” compared with the surrounding segment-specific cells (24). Moreover, it became clear that intercalated cells lacked a central cilium, at least in the cortex, which also differentiated them from the adjacent principal cells (Figure 3) (10,28–30). Their peculiar morphology was reminiscent of acid-secreting cells in the turtle bladder (31,32) and frog skin (33), and, like these cells, intercalated cells participate in urinary acid secretion, bicarbonate reabsorption, and bicarbonate secretion (5).

The question remains of how these two disparate cell types coordinate their function in these epithelia (20,34). The plasma membrane of each epithelial cell organizes into apical and basolateral domains that develop distinct membrane lipid and transport protein compositions. Thus, within a homogeneous epithelium of one cell type, the apical membranes (facing the tubular lumen) of all cells could function as a unit, and similarly for the basolateral domains of the same cells facing the interstitium. The maintenance of the apical and basolateral domains of the kidney tubular epithelium depends on the presence of functional tight junctions, the differential permeabilities of the two membranes, and the generation of gradients between the lumen and the interstitium. Recent findings have uncovered the paracrine signals that integrate the function of intercalated and principal cells in the collecting duct (35).

Three types of intercalated cells are traditionally recognized, based largely on cell morphology and localization (reviewed in reference 36). These cells fall into the categories of type A (also known as “ α ”), type B (or “ β ”), and non-A, non-B intercalated cells (Figure 2) (reviewed elsewhere [10,36]). The initial morphologic classification was upheld after both functional and immunolabeling studies once inhibitors and antibodies for individual transport proteins became available. Currently, intercalated cells are classified with respect to the presence of the chloride-bicarbonate exchanger AE1 (Slc4a1) and to the subcellular

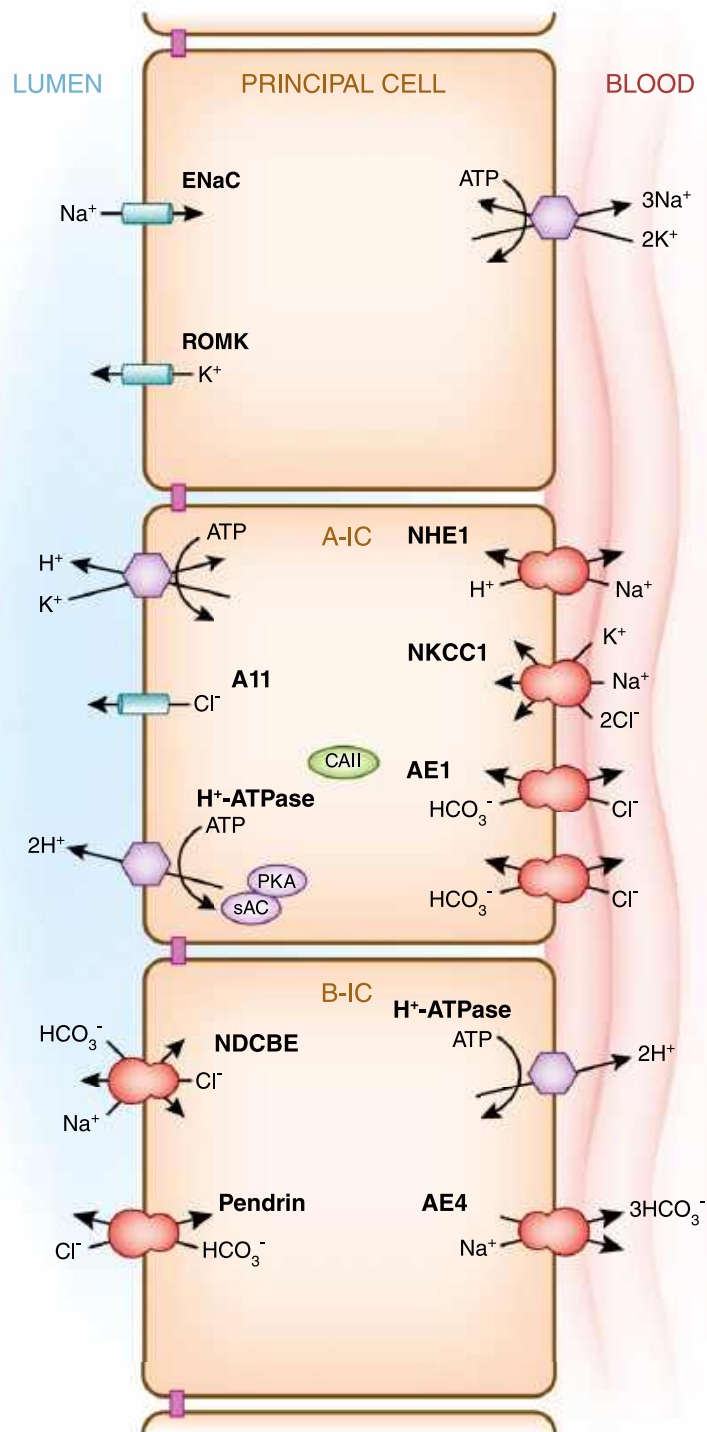


Figure 2. | Transepithelial transport processes and regulatory mechanisms in type A intercalated cells (A-IC) and type B intercalated cells (B-IC). This cartoon illustrates the major transport proteins expressed in the three main epithelial cell types present in the collecting duct: the principal cell, which expresses the epithelial sodium channel; the acid-secreting type A-IC; and type B-IC, which secretes bicarbonate while reabsorbing NaCl. In the cortical and outer medullary collecting duct, type A-ICs express H⁺-ATPase and the H⁺/K⁺-ATPase at the apical/luminal membrane, while they express the Cl⁻/HCO₃⁻ exchanger AE1 at their basolateral membrane. The bicarbonate sensor soluble adenylyl cyclase (sAC) and protein kinase A (PKA) play important roles in the regulation of the H⁺-ATPase (see Figure 5A). Slc26a11 (A11), an electrogenic Cl⁻ transporter, as well as a Cl⁻/HCO₃⁻ anion exchanger, are also expressed at the apical membrane of the type A-IC. On the other hand, the type B-ICs display an electroneutral NaCl transport/reabsorption pathway at their apical membrane that involves pendrin, a Cl⁻/HCO₃⁻ exchanger, and the Na⁺-driven Cl⁻/HCO₃⁻ exchanger (NDCBE). The proposed basolateral Na⁺ extrusion pathway would involve the cotransporter Slc4a9 (AE4). The mechanism of Cl⁻ exit remains to be elucidated. In type B-ICs, reabsorption of NaCl from the lumen is energized by the basolateral H⁺-ATPase rather than by Na⁺/K⁺-ATPase.

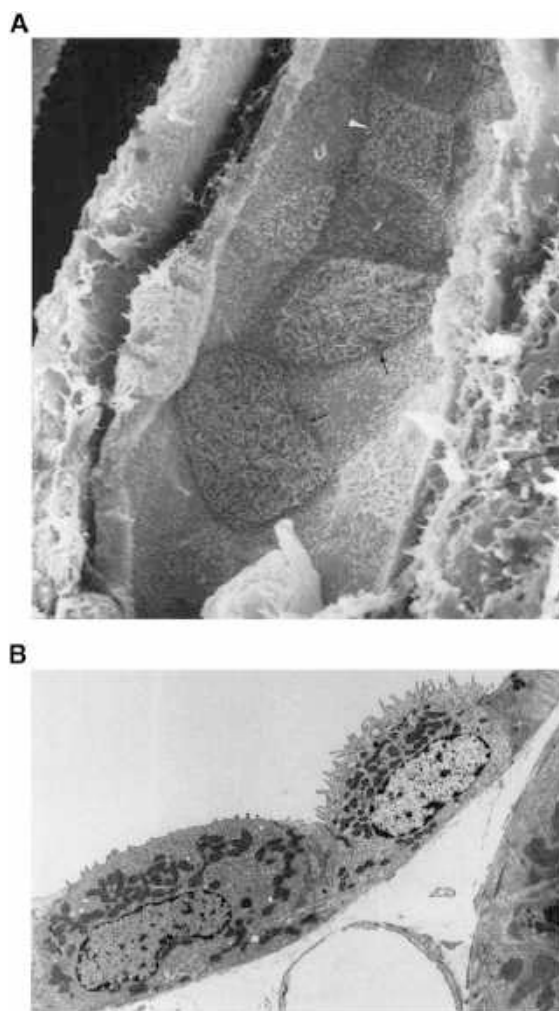


Figure 3. | Morphology of rat cortical collecting duct intercalated cells. (A) The scanning electron micrograph shows the luminal surface of the rat collecting duct. In this image principal cells can be easily identified by their small microprojections into the lumen and by the presence of a single cilium. Two configurations of intercalated cells are present in this tubule. The type A-ICs (arrows) have a large luminal surface covered mostly with microvilli, while a type B-IC (arrowhead) has a more angular cellular outline and smaller apical microvilli (original magnification, $\times 5500$). Reproduced with permission from reference 10. (B) This transmission electron micrograph of rat cortical collecting duct illustrates further the two configurations of intercalated cells. The type A-IC (right) shows a well developed apical tubulovesicular membrane compartment, with prominent microprojections that are part of the microvilli on the luminal membrane. In this image, the type B-IC (left) presents a denser cytoplasm with more abundant mitochondria and many vesicles throughout the cytoplasm. The luminal membrane of the type B cell has a quite smooth luminal membrane (original magnification, $\times 5000$). Reproduced with permission from reference 10.

localization of the multisubunit H^+ -ATPase (36,37). This classification has been further modified to reflect whether cells express the transport protein pendrin, a subtype of chloride-bicarbonate exchanger (37). Therefore, another definition for type A intercalated cells combines their function in urinary acidification, their lack of pendrin expression, the presence of H^+ -ATPase at their apical membrane, and

the expression of AE1 at their basolateral membrane. On the other hand, the intercalated cells involved in bicarbonate secretion express the chloride/bicarbonate exchanger pendrin at their apical membrane. These cells are further classified as type B and non-A, non-B intercalated cells. While type B intercalated cells express the H^+ -ATPase at their basolateral pole, the non-A, non-B intercalated cells express both the H^+ -ATPase and pendrin at their apical membrane. Moreover, non-A, non-B intercalated cells are located in the connecting segment or connecting tubule (10,38). All three types of intercalated cells express carbonic anhydrase in their cytoplasm (36,39). This metalloenzyme reversibly catalyzes the hydration of CO_2 (40) and thus leads to the formation of bicarbonate. At least 13 isoforms of carbonic anhydrase have been identified in mammals. Carbonic anhydrase II, acting in tandem with H^+ -ATPase, facilitates the coordinated proton and bicarbonate secretion from intercalated cells. Table 1 summarizes the current findings on transport or other proteins that can help in the characterization and classification of intercalated cell types.

Intercalated Cell Development and Plasticity

Interest in intercalated cell development arose, in part, because of the intriguing presence of the non-A, non-B intercalated cell, which some viewed as an intermediate type. Moreover, other intercalated cells did not fit into any of the three available classifications because of the expression of marker proteins in unexpected subcellular localizations. These other types of intercalated cells were thought to represent a series of intermediate forms that could arise if, for example, a type B intercalated cell were transforming into a type A intercalated cell under the pressure of metabolic acidosis. One such "intermediate" cell might express the H^+ -ATPase diffusely in the cytoplasm or at both the apical and basolateral domains (41,42). There is now evidence that type A and type B intercalated cell types represent different states of differentiation, as proposed by Al-Awqati and confirmed by others (16). In some of these studies carried out in the rabbit model, adaptation to acidosis was not accompanied by changes in the number of intercalated cells but rather by a change from type B to type A intercalated cells (7,43). The transition process from type B to type A intercalated cell depends on the basolateral deposition of the extracellular matrix proteins hensin (DMBT1), galectin 3, and other proteins (7,44). Overall, induction of chronic metabolic acidosis increases the proportion of type A intercalated cells while metabolic alkalosis causes an increase of type B intercalated cells (7,45,46). Moreover, mice with a hensin defect in intercalated cells indeed developed metabolic acidosis. In this mouse model, intercalated cells in the cortex had a type B phenotype (44). Interestingly, the hensin-deficient mice had modified the phenotype of medullary epithelial cells. These modified medullary cells had the ultrastructure of the type B intercalated cells usually found in cortex, while they differed from type B cells in that they did not express pendrin (47).

In addition to the above factors, the Notch signaling pathway also controls the development of primitive epithelia, such as the ones in the connecting segment and the collecting duct (20,48). The Notch signaling cascade is essential for a developmental process called lateral inhibition, where

Table 1. Relevant proteins expressed in kidney intercalated cells

Intercalated Cell Type Protein Expressed	Type A	Type B	Non-A, Non-B
Carbonic anhydrase II: catalyzes the reversible hydration of CO ₂ to bicarbonate and water	Apical	Cytoplasmic	Cytoplasmic
H ⁺ -ATPase: pumps H ⁺ across the (plasma) membrane, and into the extracellular space	Apical ^a	Basolateral ^a	Apical and diffuse vesicular ^a
AE1 (Slc4a1): anion exchanger 1, exchanges a Cl ⁻ for a bicarbonate	Basolateral ^a		
Pendrin (Slc26a4): exchanges a Cl ⁻ for a bicarbonate		Apical ^a	Apical ^a
H ⁺ /K ⁺ -ATPase: exchanges an H ⁺ for a K ⁺ at the expense of ATP	Apical	Apical?	
AE4 (Slc4a4): anion exchanger 4, exchanges a Cl ⁻ for (n) bicarbonate		Basolateral	
RhBG: Rh B glycoprotein, ammonia transporter	Basolateral		Basolateral
RhCG: Rh C Glycoprotein, ammonia transporter	Apical		Apical
NDCBE (Slc4a8): Na ⁺ -driven Cl ⁻ /bicarbonate exchanger		Apical	
A11 (Slc26a11): electrogenic Cl ⁻ transporter and Cl ⁻ /HCO ₃ ⁻ anion exchanger	Apical		
NKCC1: Na ⁺ -K ⁺ -2Cl ⁻ cotransporter	Basolateral		
BK channel or Maxi-K: K ⁺ secretion	Apical		

Adapted from reference 36. BK, big potassium.

^aThe differential localization patterns pertaining to the transport proteins identify a combination of key markers that aid in the classification of the different intercalated cell subtypes.

neighboring cells undergo one final step that gives them very different phenotypes. Notch signaling disruption indeed alters collecting duct cellular composition, resulting in more intercalated cells and fewer principal cells; in addition, probably because of the decreased numbers of principal cells, the mice also display a nephrogenic diabetes insipidus phenotype (48). The developmental events would involve ureteric bud cells differentiating into principal cells, with active Notch signaling, while intercalated cells would appear because of inactive Notch signaling (49,50). Intercalated cell development goes one step further to generate cells of type B lineage in the cortex and outer medulla. The expression of grainyhead genes results in the type B intercalated cell phenotype (21,51,52).

In rodent kidney, the appearance of intercalated cells during development has traditionally been tracked by following the expression of several of the intercalated cell-specific H⁺-ATPase subunits and other markers. This multisubunit proton pump is ubiquitous, but several of its subunits are specific to distal nephron intercalated cells (47,53). These intercalated cell-specific H⁺-ATPase subunits (such as a4 and B1) appear early in the medulla at embryonic day 15.5 (E15.5), after the detection of the expression of Foxi1, a forkhead family transcription factor that is detected in adult intercalated cells (54). Pendrin-positive intercalated cells have been detected in the mouse kidney at E14. There was also a debate on whether intercalated cells developed from principal cells or *vice versa*, or whether one cell population could contribute to the repopulation of the collecting duct after injury. Some of this controversy has been clarified by the characterization of a mouse strain lacking a transcription factor of the forkhead family, Foxi1 (55). In adult mice, Foxi1 is detected only in intercalated cells, suggesting that this transcriptional regulator is required for intercalated cell development. Indeed, Foxi1 knockout mice lack intercalated cells, and their collecting duct epithelium instead expresses a more primitive cell type with some principal and intercalated

cell characteristics. For example, this undifferentiated cell type expresses the water channel aquaporin-2, which is expressed in principal cells, and the cytosolic enzyme carbonic anhydrase II, which is used as a marker of intercalated cells. Foxi1-null mice did not express H⁺-ATPase or pendrin in their collecting ducts.

Type A Intercalated Cells, Their Major Transport Proteins, and Their Hormonal Regulation

Type A intercalated cells are present in the late distal convoluted tubule, the connecting segment, cortical and outer medullary collecting duct, and, in some reports, the early part of the inner medullary collecting duct. They are the more abundant type of intercalated cell in the outer stripe of the outer medulla in most mammalian species (10). Morphologically, they lack a cilium, they have numerous apical microvilli, and mitochondria are abundant near the apical pole (10,28). Their most characterized role is as cells that secrete protons into urine, and they can easily be identified for their lack of pendrin expression.

Type A intercalated cells can secrete H⁺ equivalents into urine *via* the H⁺-ATPase or the H⁺/K⁺-ATPase (H,K-ATPase) at their apical membrane. The latter pump exchanges one potassium ion for each extruded proton. In addition, these cells express Slc4a1, a splice variant of erythroid band 3, at the basolateral membrane (Figure 1) (42). The secretion of a proton into the tubular lumen, whether it is in exchange for potassium reabsorption or not, results in the generation of intracellular bicarbonate *via* carbonic anhydrase II, which is reabsorbed into the interstitium in exchange for chloride by AE1. The H⁺-ATPase is very abundant at the apical membrane of type A intercalated cells and in subapical vesicles or tubulovesicular structures, and they appear as 10-nm spherical structures or “studs” coating these membranes, also described as rod-shaped particles (56,57).

The H^+ -ATPase facilitates the movement of protons across the apical membrane of type A intercalated cells. Other ion movements, such as Cl^- and/or bicarbonate extrusion, compensate H^+ transport in proton-secreting cells (32,58). In the case of H^+ -ATPase, two other main factors affect its function at the plasma membrane: the pH difference across the apical membrane and the transepithelial potential difference (59). For example, this pump mediates H^+ transport at a rate that is 0 when the luminal pH is <4.5 , while the transport rate of the pump is saturated at a pH of 7.0–8.0. In addition, when the lumen potential is 120 mV relative to the interstitium, the H^+ transport rate is negligible. On the other hand, at an applied potential of -30 mV the H^+ pumping rate saturates. A hypothesis was then presented that Na^+ reabsorption through ENaC would create a more lumen negative transtubular potential difference along the distal nephron. This potential difference would favor H^+ -ATPase function in type A intercalated cells. Wagner and colleagues elucidated *in vivo* that ENaC function in the connecting segment was sufficient to induce this upregulation (60).

Both AE1 and H^+ -ATPase are upregulated in the kidney during metabolic acidosis (45), and the morphology of type A intercalated cells changes (Figure 4) (10). Although the entire collecting duct contributes to urinary acidification, the outer medullary collecting duct is very important in this process, and most intercalated cells in this segment are of type A expressing apical H^+ -ATPase (5,42). In addition, in collecting duct cells, including intercalated cells, H^+/K^+ -ATPases can be detected by following the expression

of their two α subunit isoforms: $HK\alpha_1$ (gastric) or $HK\alpha_2$ (colonic) (reviewed in reference 61). Type A intercalated cells from $HK\alpha_{1,2}$ -null mice had significantly slower acid extrusion compared with A-type intercalated cells from $HK\alpha_1$ -null or $HK\alpha_2$ -null mice (62), although the lack of either α subunit affected the rate of acid extrusion from both type A and type B cells (63).

Therefore, type A intercalated cells in the collecting duct participate in active potassium reabsorption *via* the H^+/K^+ -ATPases (Figure 2), and they undergo hypertrophy when animals are fed a low-potassium diet. However, the contribution of each α isoform in kidney potassium and acid handling remains to be determined, as the null mice for either the α_1 or α_2 subunits did not have significant changes in their acid-base or potassium excretion (62). In the setting of acidosis, the activity of H^+/K^+ -ATPase increases in the collecting duct (64,65). These findings indicate that this pump represents a key mechanism of proton secretion by the kidney in metabolic acidosis.

In addition to the H^+/K^+ -ATPase, another important regulator of K^+ homeostasis, the high-conductance big potassium (BK) or maxi-K channel is also highly expressed at the apical membrane of type A intercalated cells (66), especially in animals fed a high-potassium diet. These channels, which are less abundant in principal cells, are activated by depolarization, stretch/luminal flow, intracellular calcium increases, or hypoosmotic stress (reviewed in reference 67). Moreover, the BK channel is inhibited by the with-no-lysine kinase 4 (WNK4) in a phosphorylation-dependent manner (68,69). In a later study performed in a cell line of intercalated cell characteristics, WNK4 inhibited BK channel activity in part by increasing channel ubiquitination and degradation (70). The researchers investigating flow-induced K secretion from the BK channel in type A intercalated cells found evidence that a basolateral Na-K-Cl cotransporter (NKCC1) could support entry of K^+ into the cell, especially in light of the almost negligible levels of Na^+/K^+ -ATPase in this type of intercalated cells (Figure 2) (71).

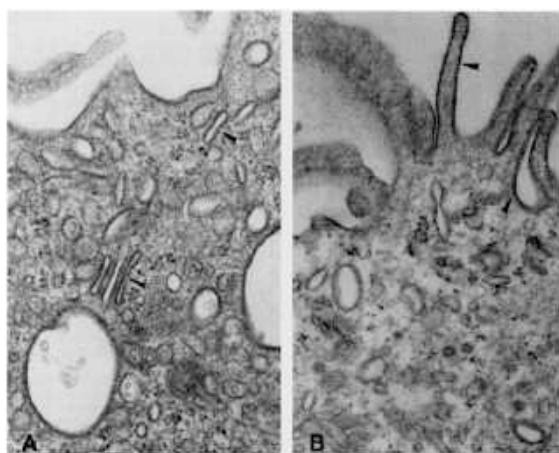


Figure 4. | Change in intercalated cell morphology in response to chronic acid-base status changes. This transmission electron micrograph shows the apical membrane of type A cells from the collecting duct from a normal rat (A) and from a rat with acute respiratory acidosis (B). In the control animal prominent studs (arrowheads) are observed on tubulovesicular structures in the control rat (A), while they are more abundant on the apical membrane in the experimental animal (B). In another study, Brown and colleagues showed that these studs are H^+ -ATPase, both at the membrane and in the tubulovesicular subapical structures (57). The increase in the number of H^+ -ATPases (studs) at the apical membrane in the animal with acidosis (B) is coupled to an increase in membrane microprojections and a decrease in the number of tubulovesicular structures in the acidotic rat (B). (Original magnification: A, $\times 48,000$; B, $\times 42,400$.) Reproduced with permission from reference 10.

Acid-Base Sensing in Intercalated Cells

The activity and subcellular localization of acid-base transport proteins can be quickly altered by changes in extracellular or intracellular pH or bicarbonate changes. These findings have led to an intense search for the pH-sensing mechanism within the intercalated cells. For example, the H^+ -ATPase in type A intercalated cells is acutely activated (within minutes to hours) by kinases such as protein kinase A (PKA), while it is acutely inhibited by the metabolic sensor AMP activated protein kinase (AMPK) (Figure 5) (72–74). This downregulation of the proton pump by AMPK is preliminary evidence that intercalated cell function can be altered when the tubule is under metabolic stress, such as under conditions of ischemia. These two kinases also regulate the H^+ -ATPase in proximal tubule and epididymis (75,76). We have characterized two major phosphorylation sites in the H^+ -ATPase A subunit; Ser-175 is required for PKA-mediated pump activation while Ser-384 is required for downregulation of the pump by AMPK (72,73). In particular, Ser-175 is in part responsible for the activation of H^+ -ATPase in endosomes downstream of G protein-coupled signaling *via* cAMP/PKA (77).

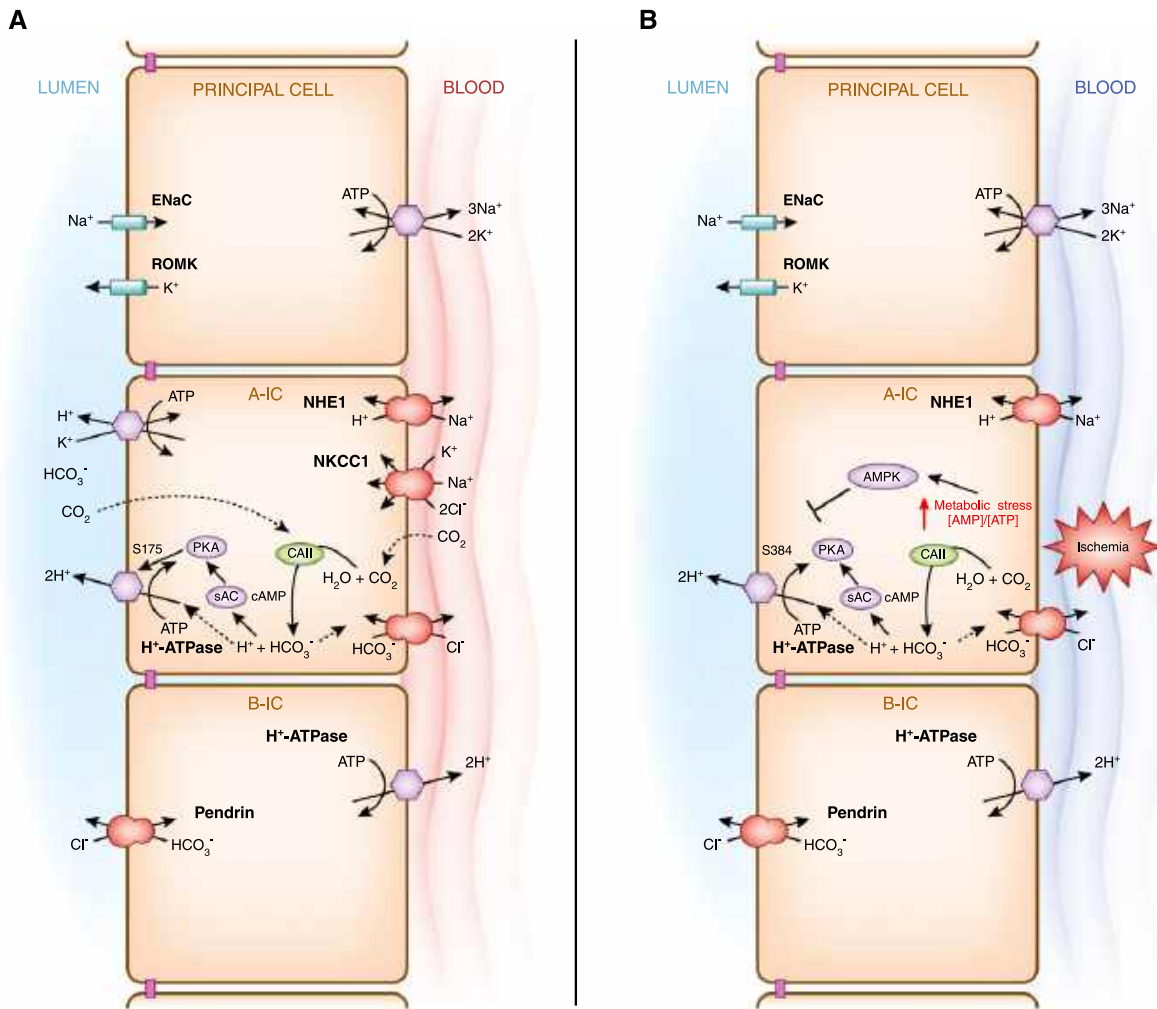


Figure 5. | Model of H⁺-ATPase coregulation at the apical membrane of type A intercalated cells by two kinases, downstream of acid-base status and of cellular metabolic stress. (A) Acute increases in the level of intracellular bicarbonate activates the bicarbonate-sensor sAC, which generates cAMP and then activates PKA. Studies using pharmacological activators have shown that exchange protein directly activated by cAMP (Epac) is not likely to play a role in H⁺-ATPase regulation (183). Carbonic anhydrase II (CAII) is involved in the generation of intracellular bicarbonate. Downstream of PKA, the A subunit of H⁺-ATPase is then phosphorylated at Ser-175 (S175) (72). This phosphorylation event is involved in activating H⁺-ATPase at the apical membrane. (B) This acute stimulatory effect of the sAC/cAMP/PKA signaling cascade on apical H⁺-ATPase activity is counterbalanced by the inhibitory effect of the metabolic sensor AMPK, downstream of ischemia or metabolic stress, for example. Acute metabolic stress leads to an elevation of the levels of AMP compared with ATP, and the increase in cellular [AMP]/[ATP] directly activates AMPK. AMPK mediates the downregulation of H⁺-ATPase activity by phosphorylating Ser-384 (S384) in the proton pump's A subunit (73).

In addition, PKA activation of the H⁺-ATPase has also been linked to the acute activation of the bicarbonate-sensor soluble adenylyl cyclase (sAC) in type A intercalated cells (Figure 5A) (74). This cascade links physiologic changes in plasma and/or intracellular bicarbonate to acute intercalated cell phenotypic changes. For example, the PKA activator cAMP directly stimulates proton secretion in primary cultures of mouse intercalated cells (78) while significantly increasing the size of apical microvilli in these cells (78). The role of bicarbonate-activated sAC in intercalated cell function is also supported in cases of increased chronic luminal bicarbonate delivery (over days) to the collecting duct (79). A recent study found that Slc26a11, recognized as an electrogenic Cl⁻ transporter as well as a Cl⁻/HCO₃⁻ anion exchanger, facilitates H⁺-ATPase function in type A intercalated cells (80).

Another important sensor of extracellular pH in the kidney is the G protein-coupled receptor 4 (GPR4). Its function was identified and characterized in cell lines in studies that used the mOMCD1 intercalated cell model (18). GPR4-null mice had decreased kidney net acid secretion and a nongap metabolic acidosis, suggesting that GPR4 is a pH sensor with a likely important role in promoting acid secretion in kidney collecting duct intercalated cells *in vivo*. Moreover, the non-receptor tyrosine kinase Pyk2 was studied as a potential sensor of pH changes in the distal tubule. Pyk2 is expressed in kidney, and it becomes autophosphorylated by several stimuli, including decreased pH (81). Studies also performed in mOMCD1 cells revealed that Pyk2 and downstream effectors increased H⁺-ATPase but not H⁺/K⁺-ATPase in this intercalated cell model.

Type B Intercalated Cells and Their Transport Processes

The classic type B intercalated cells express a chloride-bicarbonate exchanger, pendrin, at their apical membrane and express H^+ -ATPase at their basolateral membrane (Figure 6) (41,82). These cells are thought to be responsible for the secretion of OH^- equivalents. Pendrin is encoded by the SLC26A4 PDS gene. This transport protein is a member of the multifunctional sulfate transporter solute carrier 26 family (SLC26). Although it does not transport sulfate (83), it can transport anions such as iodide, Cl^- , and bicarbonate (84). Pendrin is also expressed in epithelial cells in the inner ear (85) and the thyroid, as well as in other tissues (82,86). This differential tissue expression pattern highlights different transport roles for pendrin. For example, in kidney intercalated cells and in the inner ear, pendrin acts as a chloride-bicarbonate exchanger (85), while in the thyroid gland it mediates iodide transport (86). Therefore, mutations in SLC26A4 involve various organs to differing degrees. Vaughan Pendred described this disorder as the combination of deafness and goiter (87,88). Remarkably, both patients with Pendred syndrome, which is inherited in an autosomal recessive pattern, and pendrin-null mice have normal kidney function under baseline conditions (82,89). However, upon bicarbonate administration, which induces pendrin upregulation in wild-type mice, pendrin-null mice developed a severe metabolic alkalosis. These alkali-loaded animals deficient in pendrin could not secrete bicarbonate, suggesting a critical role for pendrin in the modulation of acid-base status that protects against metabolic alkalosis (90,91).

Initially, the reabsorption of Cl^- in the collecting duct was attributed to the paracellular pathway, with some studies indicating that there was transcellular Cl^- absorption (92) (reviewed in references 4,37). Indeed, very recent studies

have confirmed that the paracellular pathway plays an important role in Cl^- reabsorption in the collecting duct (93). On the other hand, the role of type B intercalated cells in Cl^- reabsorption became clear when pendrin was identified in these cells (82). Because both sodium and chloride intake can modulate kidney function, pendrin was considered immediately as a potential mediator in the pathogenesis of hypertension (90,94). Several studies have confirmed that pendrin protein expression decreases with maneuvers that increase urinary Cl^- excretion, while protocols that caused Cl^- depletion, such as furosemide treatment, significantly increased pendrin levels. Later studies showed that metabolic acidosis induced by acetazolamide or ammonium sulfate administration reduced the levels of pendrin irrespective of low Cl^- levels in urine (95). Aldosterone and angiotensin II also increase the levels of pendrin (96,97), thus linking this transport protein to the maintenance of adequate extracellular volume and potentially to the development of hypertension. Recently, it has been proposed that pendrin may be activated *via* a kidney-specific mechanism that does not include circulating aldosterone (98). Furthermore, angiotensin II mediated pendrin upregulation and subcellular localization changes in kidney occur *via* the angiotensin 1a receptor (99). Pendrin-mediated Cl^- uptake leads to vascular volume expansion and pendrin-induced modification of ENaC abundance and function (98). Changes in luminal bicarbonate concentration and/or pH could also contribute to this signaling (100). Thus, pendrin expressed in the kidney might represent a potential target for blood pressure control. This role is illustrated in pendrin-null mice that were resistant to mineralocorticoid-induced hypertension and in turn became hypotensive when fed a low-sodium diet.

Both type B and non-A, non-B intercalated cells (Table 1) express pendrin at their apical membrane and intracellular vesicles (Figure 6) (82,101). Pendrin is more abundant

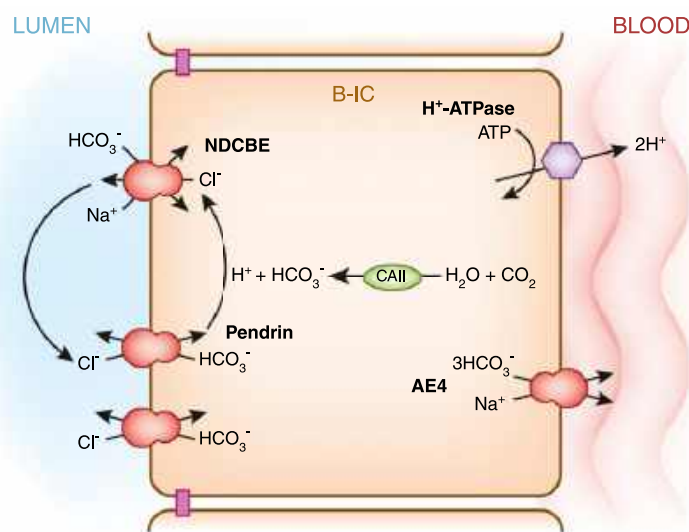


Figure 6. | Model of major transport processes and regulatory mechanisms in type B intercalated cells. This cartoon is modified from the model proposed by Chambrey and colleagues (35). These cells participate in electroneutral $NaCl$ absorption, which is energized by H^+ -ATPase. Two cycles of transport *via* pendrin, when coordinated with one cycle of NDCBE results in net uptake of one Na^+ and one Cl^- and the extrusion of two bicarbonate (HCO_3^-) ions. The Cl^- ions recycle across the apical membrane. A basolateral channel (not shown) mediates Cl^- exit. The basolateral anion exchanger 4 (AE4 or Slc4a9), together with the H^+ -ATPase also facilitates Na^+ and bicarbonate exit.

at the apical plasma membrane than intracellularly in non-A, non-B cells under baseline conditions. This finding suggests that pendrin-mediated anion exchange occurs to a greater extent in non-A, non-B cells than in type B cells (102). This difference in pendrin subcellular distribution also suggests that its function is likely regulated by trafficking between the plasma membrane and cytoplasmic vesicles. Nitric oxide also contributes to intercalated cell transport protein regulation as it reduces pendrin protein abundance in the kidney without modulating pendrin subcellular localization in a cAMP-dependent manner (103). The regulation of pendrin also involves glycosylation-dependent processes (104,105). In addition to the regulation of acid-base status, it appears that pendrin expressed in kidney intercalated cells plays a role in controlling iodide homeostasis as well, especially during high water intake (106).

Type B intercalated cells have recently come to prominence with the discovery that the Na^+ -driven chloride/bicarbonate exchanger (NDCBE) (107) and pendrin colocalize at the apical membrane of these cells (Figure 6). Together these two transport proteins mediate the until recently unexplained thiazide-sensitive electroneutral NaCl reabsorption in the collecting duct (4,11,107). Moreover, pendrin deletion in type B intercalated cells disturbs the function of ENaC in adjacent principal cells (98,100). As in intercalated cells, NaCl absorption is energized by the H^+ -ATPase and not by the Na^+/K^+ -ATPase (11); Eladari and colleagues hypothesized that Na^+ transport in type B intercalated cells was mediated by a bicarbonate-dependent transport protein at the basolateral membrane. This transport protein therefore would be energized by H^+ -ATPase. Members of the same research group had previously shown that the anion exchanger 4 (AE4/Slc4a9) was expressed basolaterally in type B intercalated cells (Figure 1) (108).

Acidosis induces important changes in the transport proteins expressed in type B intercalated cells. For example, acidosis downregulates pendrin expression at their apical membrane and in apical recycling endosomes, and moreover, AE4 expression is diminished at their basolateral pole (109).

Regulation of Intercalated Cell Transport Proteins by Hormones and Other Mediators: Effects of (Pro)renin, Renin, Angiotensin, and Aldosterone

Steroid hormone receptors, such as the mineralocorticoid receptor (MR), function downstream of the renin-angiotensin-aldosterone system (RAAS). Together with the glucocorticoid receptor these hormones elicit signaling events in the kidney that regulate salt and potassium homeostasis, intravascular volume (110), and cell metabolism (111–113). Aldosterone, *via* the MR, in turn activates ENaC in principal cells, resulting in an increase in paracellular Cl^- transport, H^+ secretion from intercalated cells, and activation of basolateral Na^+/K^+ -ATPase (reviewed in references 60,110,114). However, these transcellular transport cascades are mostly relevant to principal cells (11).

During hyperkalemia, aldosterone is also secreted. Its stimulation of ENaC *via* the MR leads to lumen electro negativity, which promotes K^+ secretion *via* the renal outer

medullary small-conductance K^+ channel (ROMK) expressed in principal cells and the flow-mediated, large-conductance, calcium-activated potassium channel or BK channel, which is expressed in intercalated cells (Figure 7) (71,115). Thus, potassium secretion is promoted without retaining sodium. It is paradoxical that activation of the same MR in the intercalated cell would result in increases of both NaCl reabsorption and regulation of acid-base transport proteins (110). The RAAS is activated during metabolic acidosis (116), while RAAS blockade inhibits kidney acid excretion in the setting of acidosis (112,117). The regulatory effects of steroid hormones on individual transport proteins in intercalated cells have been extensively studied. For example, decreased dietary potassium increases type A intercalated cell function apical projections and increases the activity of H^+/K^+ -ATPase and the BK channel (66). However, under potassium-replete conditions, which are known to increase aldosterone levels, changes in dietary sodium and serum aldosterone did not affect BK expression in intercalated cells (118).

There are several important findings to consider in the regulation of H^+ -ATPase and other transport proteins in intercalated cells by steroid hormones. For example, Wagner and colleagues showed that angiotensin II increases the expression of the H^+ -ATPase (119). Moreover, mineralocorticoids, such as aldosterone, which is released in metabolic acidosis, also increase the expression of pendrin and the H^+ -ATPase (58,97). Recently a novel modulatory pathway that affects the MR in intercalated cells has been uncovered. This pathway, which involves MR phosphorylation, is likely the key to the differential response of these cells when only aldosterone is present (as in hyperkalemia), in contrast to when both aldosterone and angiotensin II are present (as in volume depletion) (see Figure 8) (69,120). The MR can be activated by both aldosterone and cortisol (121–123). While principal cells express the enzyme 11β hydroxysteroid dehydrogenase ($11\beta\text{HSD2}$), which rapidly converts cortisol to a less active hormone, cortisone, intercalated cells lack this enzyme (69,110,121). Thus, in principal cells the MR is mostly activated by aldosterone and not cortisol, and this activation leads to the coordinated action of ENaC and the Na^+/K^+ -ATPase. A recent study elegantly showed that a single phosphorylation site, Ser-843, in the MR inactivates it, and this inactive receptor is the major species in intercalated cells (69). When there is volume depletion (Figure 8B), both angiotensin II and WNK4 promote dephosphorylation/activation of the MR. Once active, this receptor binds aldosterone and mediates the increase in H^+ -ATPase and chloride-bicarbonate exchanger activity. This coordinated action increases plasma volume while inhibiting potassium secretion. In the absence of angiotensin II, the receptor remains phosphorylated and thus aldosterone cannot promote H^+ -ATPase activity. In this scenario, aldosterone promotes K^+ secretion from principal cells. Angiotensin II also increases net transcellular chloride absorption across type B cells *via* a mechanism that involves both pendrin and activation of the H^+ -ATPase (96). Together these findings show the physiologic relevance of selective mineralocorticoid activation in intercalated cells.

The role of renin on intercalated cell function has come to the forefront because of the identification of the (Pro)renin

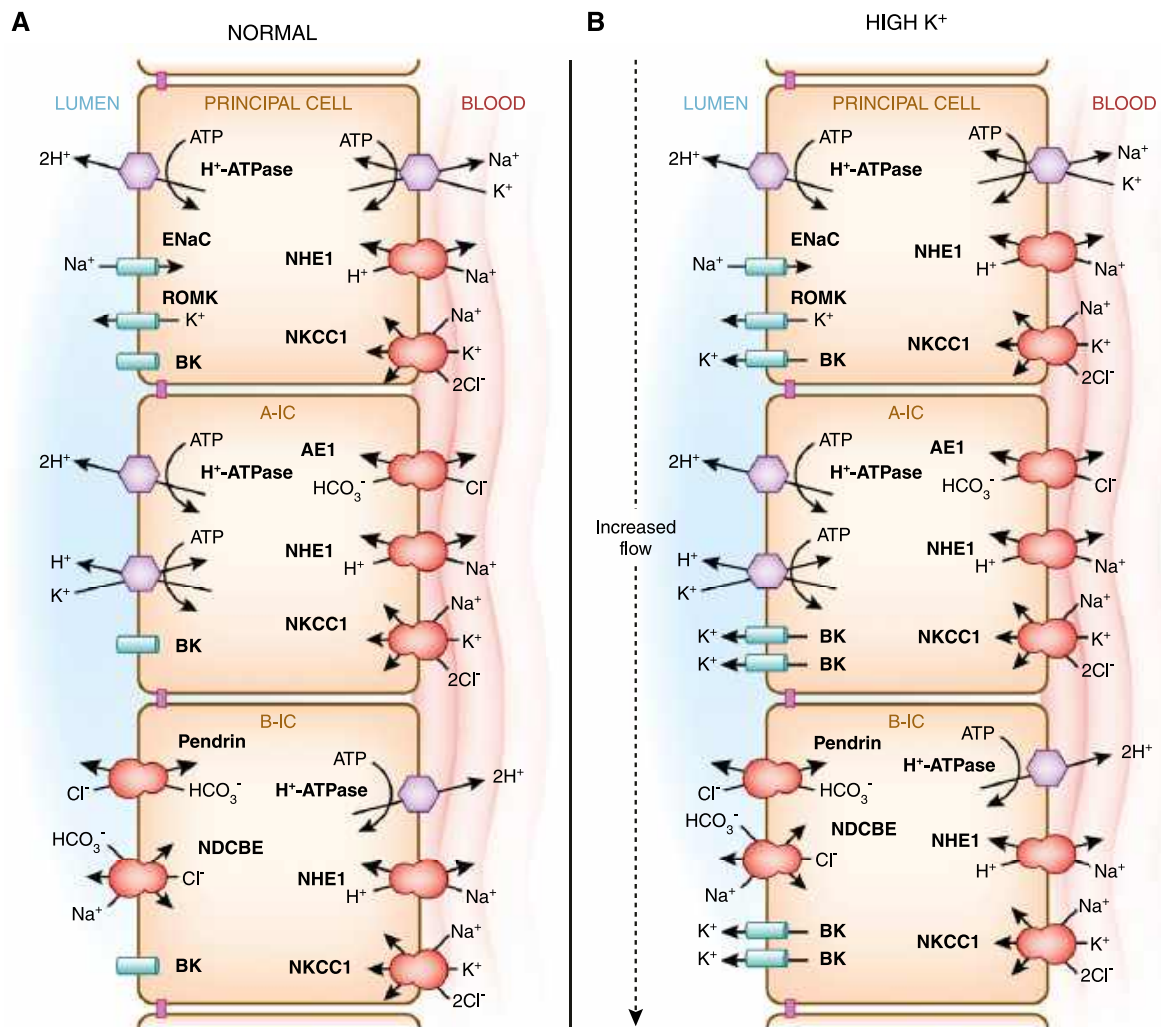


Figure 7. | Intercalated cells are involved in K^+ secretion in the collecting duct. This cartoon illustrates the location of the voltage-dependent renal outer medullary small-conductance K^+ (ROMK) and the flow-dependent big potassium (BK) channels in the intercalated cells (type A-IC and type B-IC) and principal cells. (A) Under normal dietary conditions, ROMK predominantly secretes K^+ . However, under the influence of a K^+ -rich diet, because of the increased activity of the basolateral Na^+/K^+ -ATPase, the transport via the epithelial sodium channel (ENaC)–transport increases, generating a driving force for ROMK to secrete more K^+ from the principal cells. (B) When high K^+ secretion is accompanied by increased flow, it stimulates BK channel synthesis and function in the intercalated cells (type A-IC and type B-IC).

receptor [(P)RR] as an accessory protein of H^+ -ATPase. However, and just like H^+ -ATPase, (P)RR is ubiquitously expressed. This receptor protein has a large extracellular domain that binds the enzymes renin and pro-renin (124–126). (P)RR was referred to as the ATP6AP2 (ATPase, H^+ transporting, lysosomal accessory protein 2) (127). (P)RR undergoes proteolytic cleavage to generate soluble (P)RR (128,129). (P)RR is easily detected in type A intercalated cells and not in principal cells (130,131). Binding of renin and prorenin to (P)RR increases the catalytic efficiency of these enzymes in the conversion of angiotensinogen to angiotensin by 4-fold. Prorenin secreted from the principal cells binds to the (P)RR located in the apical membrane of intercalated cells or to the soluble (P)RR, and thus enhances local angiotensin I and angiotensin II formation in the collecting duct (Figure 9) (114,132). A (P)RR interaction with the H^+ -ATPase is required for

prorenin-induced activation of Erk1/2 in a cultured cell model of intercalated cells (133). (P)RR has also been shown to play a role in vasopressin-mediated H^+ -ATPase activity and cAMP accumulation, in a prorenin-independent manner in the intercalated cells. The (P)RR has been implicated in several pathologic conditions, such as diabetic nephropathy, hypertension, albuminuria, and pre-eclampsia. However, (P)RR blockade would likely have adverse effects through its activation of H^+ -ATPase (130,134–136).

Intercalated Cell Dysfunction and the Pathogenesis of Distal Renal Tubular Acidosis

Mutations in certain intercalated cell transport proteins, or associated enzymes, can lead to distal renal tubular acidosis (dRTA) type 1, a syndrome characterized by a decreased

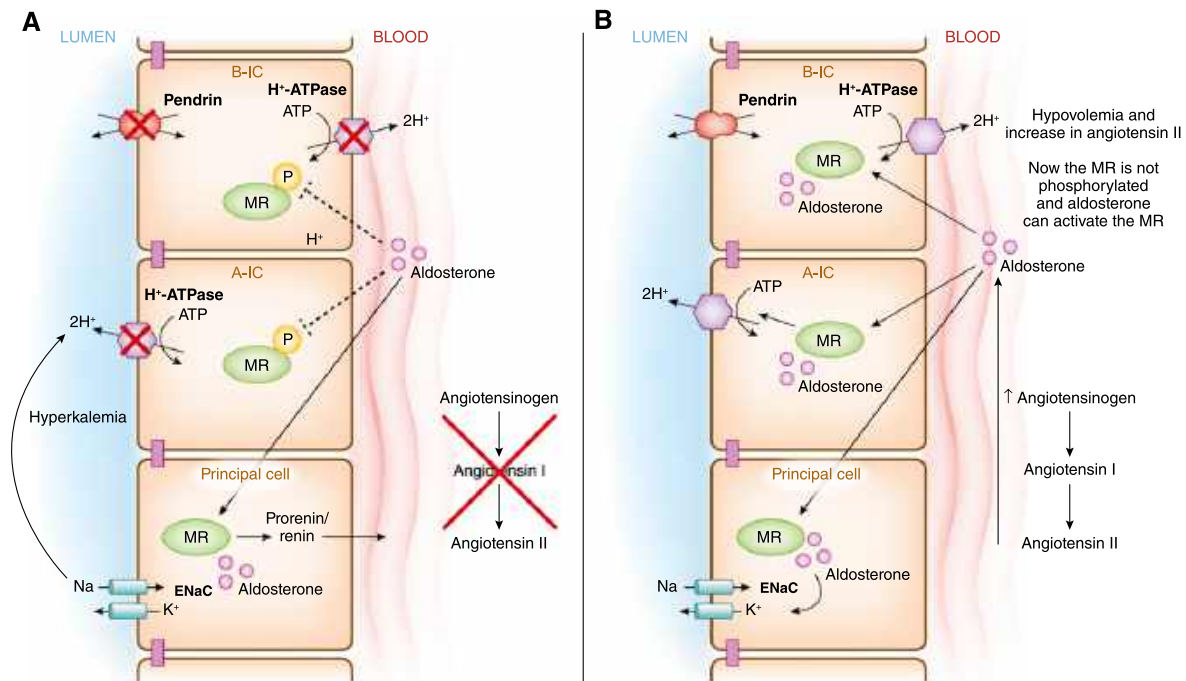


Figure 8. | Model of regulatory pathways for the mineralocorticoid receptor (MR) in intercalated cells: hyperkalemia and volume depletion. This figure depicts pathways that involve the differential phosphorylation state of the MR in principal versus intercalated cells (type A-IC; type B-IC), that are likely the key to the distinct responses of these cells in two different scenarios. (A) The first scenario involves conditions when only aldosterone is present (as in hyperkalemia). In this case, hyperkalemia leads to aldosterone secretion while no angiotensin II is present. Here, MR is phosphorylated in intercalated but not in principal cells. These conditions lead to aldosterone-mediated Na⁺ reabsorption via the epithelial sodium channel in principal cells, which drives K⁺ secretion also in principal cells. (B) In contrast, when both angiotensin II and aldosterone are present (as in intravascular volume depletion), the MR is dephosphorylated downstream of angiotensin II, and the activity of this receptor is thus restored in intercalated cells. In addition, as a result of aldosterone signaling both pendrin and H⁺-ATPase are upregulated, and in turn there is a decrease drive for K⁺ secretion. Modified from reference 69.

capability of urinary acidification and variable degrees of hyperchloremic, hypokalemic metabolic acidosis (137). When this condition is severe, the patient cannot excrete the daily acid load, which varies from 50 to 100 mEq/d depending on the patient's diet. This continued proton accumulation leads to a normal anion gap metabolic acidosis, with values of plasma bicarbonate that can fall below 10 mEq/L. The treatment involves potassium and alkali repletion. When the disease is not so severe, it is called incomplete, type 1 dRTA. These patients still have an inability to acidify the urine (pH>5.5) without a significant drop in plasma bicarbonate. Some forms of dRTA are due to a back-leak of protons resulting from the exposure of the patient to pharmacologic agents that damage the tubular plasma membrane, such as amphotericin (138). However, these patients will often display signs and symptoms associated with principal as well as intercalated cell dysfunction.

Inherited forms of dRTA most often result from the disruption of normal type A intercalated cell physiology. Patients with dRTA tend to have acidemia because of the lack of acid secretion from type A intercalated cells. This lack of acid secretion, and alkalinization of the urine, can present with nephrocalcinosis and/or nephrolithiasis, as well as hypokalemia (139). The inherited forms of dRTA have been described as having autosomal dominant or recessive modes of transmission. Some disease-causing mutations of

H⁺-ATPase $\alpha 4$ and B1 subunits result in early-onset dRTA (140–142). These findings suggest that the differential distribution of isoforms of H⁺-ATPase in intercalated versus other epithelial cells may be acquired during early infancy in humans. Another important transport protein in intercalated cells is the chloride-bicarbonate exchanger AE1. A large screen of kindreds with dRTA revealed that some mutations in AE1 cause autosomal dominant dRTA, while other defects identified in AE1 appear to result in autosomal recessive dRTA (143–145). In a mouse animal model, that deletion of carbonic anhydrase II reduces the number of intercalated cells in both type A and type B intercalated cells (146).

In humans, dRTA is often accompanied by some degree of salt, water, and potassium losses, and the causes for these findings were not well understood (147). Recently, a mouse model of dRTA, which was deficient in the intercalated cell-specific B1 subunit of H⁺-ATPase (ATP6V1B1), also presented with urinary losses, leading to hypovolemia, hypokalemia, and polyuria (Figure 10) (35). Although these mice did not present with acidosis unless challenged with an acid load (148), the molecular defect in H⁺-ATPase led to dysfunction of both ENaC and the combined pendrin/NDCBE (35). Researchers studying this mouse model discovered that the NaCl urinary loss coincided with increased prostaglandin E₂ (PGE₂) and ATP levels in urine. PGE₂ is a known inhibitor of Na⁺ transport in the collecting duct (149–151).

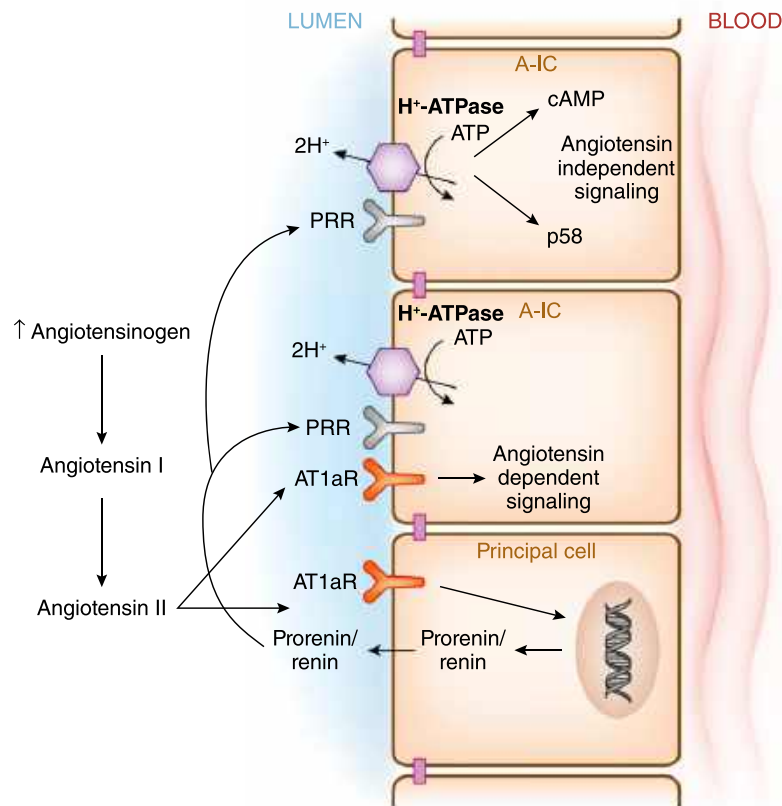


Figure 9. | Intercalated cells are targets of paracrine angiotensin II signaling. Angiotensin II stimulates secretion of prorenin and renin from principal cells. Prorenin binds to the prorenin receptor at the apical membrane of type A-IC, where in turn it stimulates the synthesis of angiotensin II from angiotensin I via the action of angiotensinogen, which originates in proximal segments of the nephron. Angiotensin II then binds to the angiotensin receptor 1a (AT1aR) in the type A-IC. Moreover, prorenin binds to its receptor in type A-IC to stimulate signaling via p58 or cAMP.

Indeed, when PGE₂ synthesis was blocked *in vivo*, the animals displayed restored ENaC levels in the cortex (35). This elegant study proposed a mechanism wherein type B intercalated cells would sense increases in NaCl delivery to the distal nephron, leading to release of ATP from intracellular stores. This ATP release would be followed by an increase in PGE₂ from the type B intercalated cells, and then to a decrease in Na⁺ absorption by the adjacent principal cells.

In addition, rare genetic defects, such as carbonic anhydrase II deficiency, can lead to intercalated cell as well as proximal tubular dysfunction. This renal tubular acidosis is also referred to as type 3 or mixed renal tubular acidosis (137,152). The patients with this genetic defect also present with osteopetrosis and cerebral calcification.

Acquired Intercalated Cell Dysfunction

Amphotericin can cause a generalized dysfunction of the kidney tubule, including intercalated cells, because it damages the plasma membrane and causes an H⁺ back-leak of protons due to the exposure (138,153). On the other hand, some conditions, such as Sjögren syndrome and some cases of systemic lupus erythematosus, that can present with dRTA due to more predominant intercalated cell

dysfunction. The estimates of patients with Sjögren syndrome and dRTA vary from 30% to 70%; this condition often presents in the context of tubulointerstitial nephritis (154–156). Autoimmune antibodies isolated from patients interact with the intercalated cells, but the precise antigens have not been determined (157). Kidney biopsies of patients with Sjögren syndrome and dRTA reveal a reduced expression of H⁺-ATPase and AE1 (158,159). There is also decrease in the total number of intercalated cells in these patients. Taken together, Sjögren syndrome is associated with loss of intercalated cell transport activity associated with defects in acid-base balance, although the precise events in the development of these findings are yet to be explored.

Lithium is a proven treatment for bipolar disorder, and approximately 40% of patients receiving this treatment develop urinary concentrating defects and eventually nephrogenic diabetes insipidus, which can contribute to ESRD (reviewed in references 160,161). One of the many renal manifestations of long-term lithium treatment is that patients develop an incomplete form of renal tubular acidosis that is usually not clinically relevant (162). Despite this clinical syndrome, the findings in rats treated with lithium reveal increased numbers of type A intercalated cells that are present even in the inner medulla (163). It has been proposed that lithium decreases the gradient for proton secretion

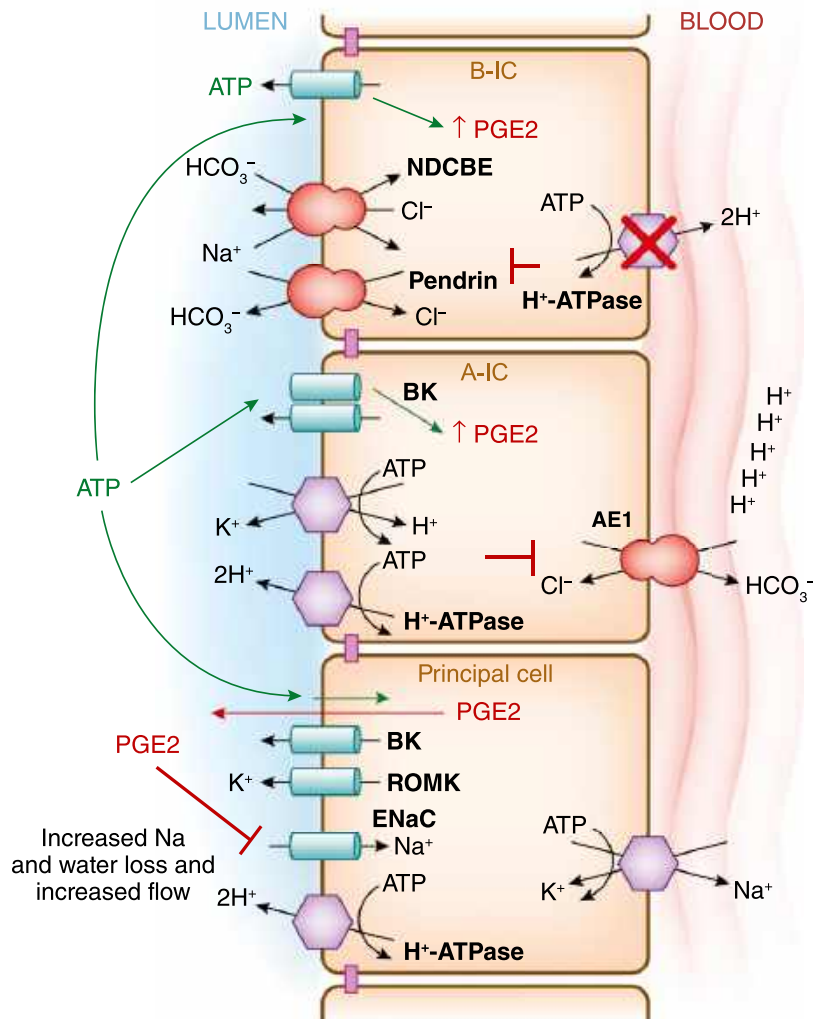


Figure 10. | Intercalated cells are necessary to maintain body fluid and electrolyte balance. This model summarizes the recent findings by Gueutin and colleagues (35), which showed how H⁺-ATPase dysfunction in type B-IC leads to the urinary water and sodium losses in patients with distal renal tubule acidosis. This group studied mice with significantly decreased H⁺-ATPase activity in the collecting duct intercalated cells due to knockout of the B1 of the H⁺-ATPase subunit (ATP6V1B1^{-/-} mice). The specific knockout of the B1 subunit does not disturb H⁺-ATPase expression in the proximal tubule. These animals presented with a significant urinary loss of NaCl, revealing impaired function of epithelial sodium channel (ENaC) in principal cells, as well as decreased pendrin and NDCBE function in type B-IC in the cortical collecting duct. These animals had an upregulation of ENaC in the medulla. High levels of prostaglandin E₂ (PGE2) and ATP were detected in the urine of these animals. When PGE2 was normalized using pharmacologic agents these animals also normalized their ENaC levels in the cortex, and they had improved polyuria and hypokalemia. When the H⁺-ATPase was inactivated in type B-IC, it resulted in ATP release with the subsequent increases in PGE2 release from these cell as well.

(a voltage-dependent defect) along with a concomitant decrease in H⁺-ATPase activity. Therefore, the observed increase in the number of intercalated cells may be a potential defense mechanism against the initial mechanism of lithium-induced acidosis. Moreover, lithium inhibits glycogen synthase 3 β (reviewed in reference 164). Inhibition of this kinase during kidney development induces nephron differentiation *via* the Wnt signaling pathway (165). Wnt signaling, a very relevant cascade needed for normal embryonic development, has been reviewed elsewhere (166). Therefore, another explanation for the increased number of intercalated cells seen with lithium treatment could be the disruption of Wnt signaling downstream of lithium-mediated glycogen synthase kinase 3 β inhibition.

Findings similar to those observed with long-term lithium treatment were described after dietary K⁺ depletion, with increases in intercalated cells that were reversed upon dietary K⁺ repletion (167). These researchers concluded that principal cells and intercalated cells may interconvert in response to changes in dietary K⁺ and that autophagy was likely involved in the pathways driving this interconversion.

The antirejection medications cyclosporine and tacrolimus affect intercalated cell function and result in the development of dRTA, numerous changes in proximal and distal nephron epithelial transport proteins, and a decrease in the non-type A intercalated cells (168,169). Cyclosporine also inhibits cyclophilin and thus affects hensen polymerization after

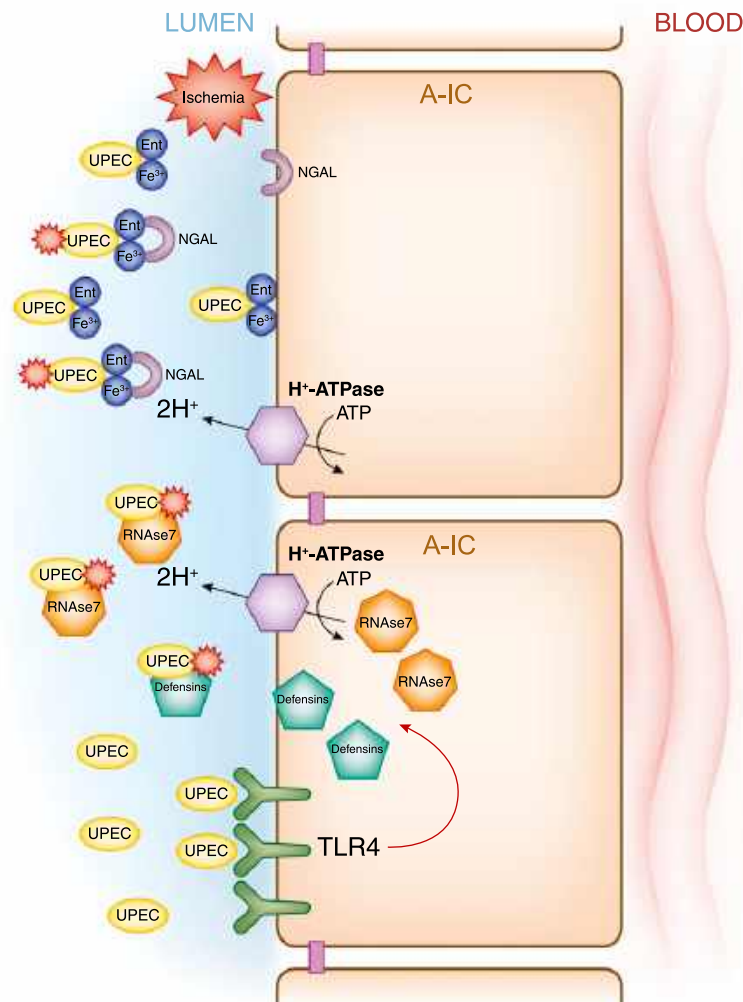


Figure 11. | The intercalated cells as key effectors of the innate immune system. This model presents various signaling cascades triggered in intercalated cells by pathologic conditions that can result in the release of defensins. Collecting duct epithelial cells respond to gram-negative bacteria (uropathogenic *Escherichia coli* [UPEC]) by activating cascades downstream from the toll-like receptor 4 (TLR4) and some TLR4-independent pathways (lower). Ischemic injury also induces release of the defensin neutrophil gelatinase-associated lipocalin from intercalated cells (NGAL) (upper), and type A-IC also secrete the defensin RNase7, which is a key molecule to prevent urinary tract infection. Functional apical H^+ -ATPase is also relevant for the type A intercalated cell anti-microbial function.

extracellular deposition, a step that when defective reduces terminal differentiation of intercalated cells (170).

Intercalated Cells and the Innate Immune Response

The epithelial cells lining the urinary tract play a major role in maintaining the sterility of urine, which is achieved by mucus production, urinary flow, and bladder emptying, all of which provide immediate defense against microbial invasion. This innate defense cascade is less specific than the adaptive immune response. Among the better-known components of the innate immune response are the Toll-like receptors (TLRs). TLRs play a key role in containing urinary tract infections and pyelonephritis. More than 13 TLRs have been identified in mammals, and kidney tubular epithelial cells express most of them. Among these TLRs, TLR4 has been implicated in the innate immune defense against uropathogenic *Escherichia coli*, the most frequent pathogen responsible for urinary tract infection (171). TLR4, which recognizes

lipopolysaccharides in the outer membrane of gram-negative bacteria, is expressed in mouse collecting duct intercalated cells (172). TLRs induce a dynamic immune response *via* several signaling pathways, including the production of antimicrobial peptides (AMPs) (Figure 11) (172,173).

AMPs are polypeptides of <100 amino acids that at physiologic concentrations are active against bacteria, enveloped viruses, fungi, and protozoa. AMPs are expressed by neutrophils or epithelial cells constitutively or after induction by exposure of the cells to pathogens (174). Urinary tract and kidney epithelial cells synthesize several AMPs, including defensins, cathelicidin, hepcidin, and ribonuclease 7 (RNase7). Among these AMPs, defensins and RNase7 are expressed in the kidney collecting duct (175). Defensins, which have broad-spectrum antimicrobial activity, are classified into two main subfamilies: α - and β -defensins. Of the β -defensins, human β -defensin-1 and β -defensin-2 are expressed in the loop of Henle, distal tubule, and collecting duct. In contrast to human β -defensin-1,

human β -defensin-2 is not constitutively expressed in the kidney, but instead its upregulation has been implicated in the immune response during chronic kidney infection (176). On the other hand, RNase7 is a potent AMP constitutively expressed by the uroepithelium of the bladder, ureter, and intercalated cells. Urinary concentrations of RNase7 are much greater than those of other AMPs, exhibiting a bactericidal activity against both gram-negative and gram-positive bacteria.

Another process that implicates type A intercalated cells in the immune response during urinary tract infection is that these cells secrete the bacteriostatic protein lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL). An important difference between NGAL and other AMPs is that NGAL expression is intensively upregulated by both septic and aseptic causes of AKI (177,178). Barasch and colleagues showed that type A intercalated cells are key elements in the production of this bacteriostatic protein. They used a mouse model expressing an NGAL reporter gene, which showed NGAL expression after kidney ischemia (179,180). In contrast to most of the AMPs, NGAL is specific in its binding to enterochelin (Ent), a product of gram-negative bacteria that removes Fe^{3+} from transferrin (181,182). When NGAL is secreted, it binds the Ent: Fe^{3+} complex and thus it exerts its bacteriostatic effects (181,182). In addition to its effects on Ent, NGAL secretion is required for TLR4 to undergo activation (179). Moreover, mice that lack type A intercalated cells have reduced urinary acidification and lipocalin 2/NGAL secretion into urine, as well as decreased clearance of bacteria from urine (179). The importance of intercalated cells in the innate immune response is highlighted by the recent characterization of yet another scavenger receptor called secretory glycoprotein 5D-SRCRB derived from these cells (183).

Summary and Conclusions

When intercalated cells were first identified, their morphology was distinctive and reminiscent of acid-secreting cells in more primitive epithelia. Since the 1960s, the essential role of the intercalated cells in acid-base homeostasis was solidified, first with the characterization of the transport processes in the distal nephron and later with the identification of individual membrane transport proteins, such as H^+ -ATPase, AE1, and pendrin, contributing to those processes. The role of intercalated cells in the pathogenesis of distal (type 1) and mixed renal tubule acidosis has been clearly established. In the last few years, the acid-base cellular sensors such as sAC and GPR4, and downstream-activated kinases such as PKA or the metabolic sensor AMPK have been recognized as key regulators of intercalated cell function. Moreover, the role of intercalated cells as regulators of salt reabsorption and intravascular volume preservation was established with the identification of NDCBE and pendrin in type B intercalated cells. Lately, it has come to the forefront that both intercalated cell volume regulation and the functional integrity of the distal nephron depend on H^+ -ATPase function in these fascinating cells.

Acknowledgments

We thank Dr. Kenneth R. Hallows for his critical reading of the manuscript and Dr. Arohan Subramanya for helpful suggestions. We thank Mr. Andrew P. Zerby for bibliography research support.

This work was supported in part by the National Institutes of Health grant R01-DK084184 and P30-DK079307, as well as by a Junior Scholar Award of the Department of Medicine at the University of Pittsburgh School of Medicine (N.M.P.-S.) and F32-DK097889 (M.M.A.).

Disclosures

Recent funding was received from Sanofi (for a postdoctoral fellowship grant to M.M.A.).

References

1. Crayen ML, Thoenes W: Architecture and cell structures in the distal nephron of the rat kidney. *Cytobiologie* 17: 197–211, 1978
2. Davies JA, Davey MG: Collecting duct morphogenesis. *Pediatr Nephrol* 13: 535–541, 1999
3. Meneton P, Loffing J, Warnock DG: Sodium and potassium handling by the aldosterone-sensitive distal nephron: The pivotal role of the distal and connecting tubule. *Am J Physiol Renal Physiol* 287: F593–F601, 2004
4. Eladari D, Chambrey R, Peti-Peterdi J: A new look at electrolyte transport in the distal tubule. *Annu Rev Physiol* 74: 325–349, 2012
5. Madsen KM, Tisher CC: Structural-functional relationship along the distal nephron. *Am J Physiol* 250: F1–F15, 1986
6. Smith H: *The Kidney: Structure and function in Health and Disease*, Oxford, Oxford University Press, 1951
7. Al-Awqati Q: Terminal differentiation in epithelia: The role of integrins in hensin polymerization. *Annu Rev Physiol* 73: 401–412, 2011
8. Rodríguez Soriano J: Renal tubular acidosis: The clinical entity. *J Am Soc Nephrol* 13: 2160–2170, 2002
9. Weiner ID, Mitch WE, Sands JM: Urea and ammonia metabolism and the control of renal nitrogen excretion [published online ahead of print July 30, 2014]. *Clin J Am Soc Nephrol* doi: 10.2215/CJN.10311013
10. Madsen KM, Verlander JW, Tisher CC: Relationship between structure and function in distal tubule and collecting duct. *J Electron Microscop Tech* 9: 187–208, 1988
11. Chambrey R, Kurth I, Peti-Peterdi J, Houillier P, Purkerson JM, Levie F, Hentschke M, Zdebek AA, Schwartz GJ, Hübner CA, Eladari D: Renal intercalated cells are rather energized by a proton than a sodium pump. *Proc Natl Acad Sci U S A* 110: 7928–7933, 2013
12. Kaissling B, Kriz W: Structural analysis of the rabbit kidney. *Adv Anat Embryol Cell Biol* 56: 1–123, 1979
13. Edwards JC, van Adelsberg J, Rater M, Herzlinger D, Lebowitz J, al-Awqati Q: Conditional immortalization of bicarbonate-secreting intercalated cells from rabbit. *Am J Physiol* 263: C521–C529, 1992
14. Guntupalli J, Onuigbo M, Wall S, Alpern RJ, DuBose TD Jr: Adaptation to low- K^+ media increases H^+ - K^+ -ATPase but not H^+ -ATPase-mediated pH recovery in OMCD1 cells. *Am J Physiol* 273: C558–C571, 1997
15. Gekle M, Wünsch S, Oberleithner H, Silbernagl S: Characterization of two MDCK-cell subtypes as a model system to study principal cell and intercalated cell properties. *Pflügers Arch* 428: 157–162, 1994
16. Al-Awqati Q: Plasticity in epithelial polarity of renal intercalated cells: Targeting of the H^+ -ATPase and band 3. *Am J Physiol* 270: C1571–C1580, 1996
17. van Adelsberg J, Edwards JC, Takito J, Kiss B, al-Awqati Q: An induced extracellular matrix protein reverses the polarity of band 3 in intercalated epithelial cells. *Cell* 76: 1053–1061, 1994
18. Sun X, Yang LV, Tiegs BC, Arend LJ, McGraw DW, Penn RB, Petrovic S: Deletion of the pH sensor GPR4 decreases renal acid excretion. *J Am Soc Nephrol* 21: 1745–1755, 2010
19. Schwartz JH, Li G, Yang Q, Suri V, Ross JJ, Alexander EA: Role of SNAREs and H^+ -ATPase in the targeting of proton pump-coated vesicles to collecting duct cell apical membrane. *Kidney Int* 72: 1310–1315, 2007
20. Sampogna RV, Al-Awqati Q: Salt and pepper distribution of cell types in the collecting duct. *J Am Soc Nephrol* 24: 163–165, 2013

21. Al-Awqati Q, Gao XB: Differentiation of intercalated cells in the kidney. *Physiology (Bethesda)* 26: 266–272, 2011
22. Wieczorek H, Brown D, Grinstein S, Ehrenfeld J, Harvey WR: Animal plasma membrane energization by proton-motive V-ATPases. *BioEssays* 21: 637–648, 1999
23. Liu W, Xu S, Woda C, Kim P, Weinbaum S, Satlin LM: Effect of flow and stretch on the $[Ca^{2+}]_i$ response of principal and intercalated cells in cortical collecting duct. *Am J Physiol Renal Physiol* 285: F998–F1012, 2003
24. Griffith LD, Bulger RE, Trump BF: Fine structure and staining of mucosubstances on “intercalated cells” from the rat distal convoluted tubule and collecting duct. *Anat Rec* 160: 643–662, 1968
25. Hancox NM, Komender J: Quantitative and qualitative changes in the “dark” cells of the renal collecting tubules in rats deprived of water. *Q J Exp Physiol Cogn Med Sci* 48: 346–354, 1963
26. Clark SL Jr: Cellular differentiation in the kidneys of newborn mice studies with the electron microscope. *J Biophys Biochem Cytol* 3: 349–362, 1957
27. Flume JB, Ashworth CT, James JA: An electron microscopic study of tubular lesions in human kidney biopsy specimens. *Am J Pathol* 43: 1067–1087, 1963
28. Latta H, Maunsbach AB, Madden SC: Cilia in different segments of the rat nephron. *J Biophys Biochem Cytol* 11: 248–252, 1961
29. Pfaller W, Klima J: A critical reevaluation of the structure of the rat uriniferous tubule as revealed by scanning electron microscopy. *Cell Tissue Res* 166: 91–100, 1976
30. Schwartz EA, Leonard ML, Bizios R, Bowser SS: Analysis and modeling of the primary cilium bending response to fluid shear. *Am J Physiol* 272: F132–F138, 1997
31. Stetson DL, Steinmetz PR: Alpha and beta types of carbonic anhydrase-rich cells in turtle bladder. *Am J Physiol* 249: F553–F565, 1985
32. Schwartz JH, Steinmetz PR: CO₂ requirements for H⁺ secretion by the isolated turtle bladder. *Am J Physiol* 220: 2051–2057, 1971
33. Harvey BJ: Energization of sodium absorption by the H⁺(+)-ATPase pump in mitochondria-rich cells of frog skin. *J Exp Biol* 172: 289–309, 1992
34. Kleyman TR, Satlin LM, Hallows KR: Opening lines of communication in the distal nephron. *J Clin Invest* 123: 4139–4141, 2013
35. Gueutin V, Vallet M, Jayat M, Peti-Peterdi J, Cornière N, Leviel F, Sohet F, Wagner CA, Eladari D, Chambrey R: Renal β -intercalated cells maintain body fluid and electrolyte balance. *J Clin Invest* 123: 4219–4231, 2013
36. Kriz W, Kaissl B: Structural Organization of the Mammalian Kidney. In: *Seldin and Giebisch's The Kidney: Physiology and Pathophysiology*, edited by Alpern RJ, Caplan MJ, Moe OW, London, Academic Press, 2013, pp 595–691
37. Wall SM, Weinstein AM: Cortical distal nephron Cl⁻ transport in volume homeostasis and blood pressure regulation. *Am J Physiol Renal Physiol* 305: F427–F438, 2013
38. Kim J, Kim YH, Cha JH, Tisher CC, Madsen KM: Intercalated cell subtypes in connecting tubule and cortical collecting duct of rat and mouse. *J Am Soc Nephrol* 10: 1–12, 1999
39. Spicer SS, Stoward PJ, Tashian RE: The immunohistochemical localization of carbonic anhydrase in rodent tissues. *J Histochem Cytochem* 27: 820–831, 1979
40. Schwartz GJ: Physiology and molecular biology of renal carbonic anhydrase. *J Nephrol* 15[Suppl 5]: S61–S74, 2002
41. Brown D, Hirsch S, Gluck S: An H⁺-ATPase in opposite plasma membrane domains in kidney epithelial cell subpopulations. *Nature* 331: 622–624, 1988
42. Alper SL, Natale J, Gluck S, Lodish HF, Brown D: Subtypes of intercalated cells in rat kidney collecting duct defined by antibodies against erythroid band 3 and renal vacuolar H⁺-ATPase. *Proc Natl Acad Sci U S A* 86: 5429–5433, 1989
43. Purkerson JM, Tsuruoka S, Suter DZ, Nakamori A, Schwartz GJ: Adaptation to metabolic acidosis and its recovery are associated with changes in anion exchanger distribution and expression in the cortical collecting duct. *Kidney Int* 78: 993–1005, 2010
44. Gao X, Eladari D, Leviel F, Tew BY, Miró-Julà C, Cheema FH, Miller L, Nelson R, Paunescu TG, McKee M, Brown D, Al-Awqati Q: Deletion of hensen/DMBT1 blocks conversion of beta- to alpha-intercalated cells and induces distal renal tubular acidosis. *Proc Natl Acad Sci U S A* 107: 21872–21877, 2010
45. Bastani B, Purcell H, Hemken P, Trigg D, Gluck S: Expression and distribution of renal vacuolar proton-translocating adenosine triphosphatase in response to chronic acid and alkali loads in the rat. *J Clin Invest* 88: 126–136, 1991
46. Sabolić I, Brown D, Gluck SL, Alper SL: Regulation of AE1 anion exchanger and H⁺(+)-ATPase in rat cortex by acute metabolic acidosis and alkalosis. *Kidney Int* 51: 125–137, 1997
47. Forgac M: Vacuolar ATPases: Rotary proton pumps in physiology and pathophysiology. *Nat Rev Mol Cell Biol* 8: 917–929, 2007
48. Jeong HW, Jeon US, Koo BK, Kim WY, Im SK, Shin J, Cho Y, Kim J, Kong YY: Inactivation of Notch signaling in the renal collecting duct causes nephrogenic diabetes insipidus in mice. *J Clin Invest* 119: 3290–3300, 2009
49. Chen L, Al-Awqati Q: Segmental expression of Notch and Hairy genes in nephrogenesis. *Am J Physiol Renal Physiol* 288: F939–F952, 2005
50. Liu Y, Pathak N, Kramer-Zucker A, Drummond IA: Notch signaling controls the differentiation of transporting epithelia and multiciliated cells in the zebrafish pronephros. *Development* 134: 1111–1122, 2007
51. Yamaguchi Y, Yonemura S, Takada S: Grainyhead-related transcription factor is required for duct maturation in the salivary gland and the kidney of the mouse. *Development* 133: 4737–4748, 2006
52. Quigley IK, Stubbs JL, Kintner C: Specification of ion transport cells in the *Xenopus* larval skin. *Development* 138: 705–714, 2011
53. Breton S, Brown D: Regulation of luminal acidification by the V-ATPase. *Physiology (Bethesda)* 28: 318–329, 2013
54. Vidarsson H, Westergren R, Heglin M, Blomqvist SR, Breton S, Enerbäck S: The forkhead transcription factor Foxi1 is a master regulator of vacuolar H⁺-ATPase proton pump subunits in the inner ear, kidney and epididymis. *PLoS ONE* 4: e4471, 2009
55. Blomqvist SR, Vidarsson H, Fitzgerald S, Johansson BR, Ollerstam A, Brown R, Persson AE, Bergström GG, Enerbäck S: Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. *J Clin Invest* 113: 1560–1570, 2004
56. Brown D, Weyer P, Orci L: Nonclathrin-coated vesicles are involved in endocytosis in kidney collecting duct intercalated cells. *Anat Rec* 218: 237–242, 1987
57. Brown D, Gluck S, Hartwig J: Structure of the novel membrane-coating material in proton-secreting epithelial cells and identification as an H⁺-ATPase. *J Cell Biol* 105: 1637–1648, 1987
58. Wagner CA, Finberg KE, Breton S, Marshansky V, Brown D, Geibel JP: Renal vacuolar H⁺-ATPase. *Physiol Rev* 84: 1263–1314, 2004
59. Andersen OS, Silveira JE, Steinmetz PR: Intrinsic characteristics of the proton pump in the luminal membrane of a tight urinary epithelium. The relation between transport rate and $\Delta\mu$ H. *J Gen Physiol* 86: 215–234, 1985
60. Kovacicova J, Winter C, Löffing-Cueni D, Löffing J, Finberg KE, Lifton RP, Hummler E, Rossier B, Wagner CA: The connecting tubule is the main site of the furosemide-induced urinary acidification by the vacuolar H⁺-ATPase. *Kidney Int* 70: 1706–1716, 2006
61. Gumz ML, Lynch IJ, Greenlee MM, Cain BD, Wingo CS: The renal H⁺-K⁺-ATPases: physiology, regulation, and structure. *Am J Physiol Renal Physiol* 298: F12–F21, 2010
62. Lynch IJ, Rudin A, Xia SL, Stow LR, Shull GE, Weiner ID, Cain BD, Wingo CS: Impaired acid secretion in cortical collecting duct intercalated cells from H-K-ATPase-deficient mice: role of HKalpha isoforms. *Am J Physiol Renal Physiol* 294: F621–F627, 2008
63. Greenlee MM, Lynch IJ, Gumz ML, Cain BD, Wingo CS: The renal H,K-ATPases. *Curr Opin Nephrol Hypertens* 19: 478–482, 2010
64. Silver RB, Frindt G, Mennitt P, Satlin LM: Characterization and regulation of H-K-ATPase in intercalated cells of rabbit cortical collecting duct. *J Exp Zool* 279: 443–455, 1997
65. Silver RB, Mennitt PA, Satlin LM: Stimulation of apical H-K-ATPase in intercalated cells of cortical collecting duct with chronic metabolic acidosis. *Am J Physiol* 270: F539–F547, 1996

66. Najjar F, Zhou H, Morimoto T, Bruns JB, Li HS, Liu W, Kleyman TR, Satlin LM: Dietary K⁺ regulates apical membrane expression of maxi-K channels in rabbit cortical collecting duct. *Am J Physiol Renal Physiol* 289: F922–F932, 2005
67. Woda CB, Miyawaki N, Ramalakshmi S, Ramkumar M, Rojas R, Zavilowitz B, Kleyman TR, Satlin LM: Ontogeny of flow-stimulated potassium secretion in rabbit cortical collecting duct: functional and molecular aspects. *Am J Physiol Renal Physiol* 285: F629–F639, 2003
68. Zhuang J, Zhang X, Wang D, Li J, Zhou B, Shi Z, Gu D, Denson DD, Eaton DC, Cai H: WNK4 kinase inhibits Maxi K channel activity by a kinase-dependent mechanism. *Am J Physiol Renal Physiol* 301: F410–F419, 2011
69. Shibata S, Rinehart J, Zhang J, Moeckel G, Castañeda-Bueno M, Stiegler AL, Boggon TJ, Gamba G, Lifton RP: Mineralocorticoid receptor phosphorylation regulates ligand binding and renal response to volume depletion and hyperkalemia. *Cell Metab* 18: 660–671, 2013
70. Wang Z, Subramanya AR, Satlin LM, Pastor-Soler NM, Carattino MD, Kleyman TR: Regulation of large-conductance Ca²⁺-activated K⁺ channels by WNK4 kinase. *Am J Physiol Cell Physiol* 305: C846–C853, 2013
71. Palmer LG, Frindt G: High-conductance K channels in intercalated cells of the rat distal nephron. *Am J Physiol Renal Physiol* 292: F966–F973, 2007
72. Alzamora R, Thali RF, Gong F, Smolak C, Li H, Baty CJ, Bertrand CA, Auchli Y, Brunisholz RA, Neumann D, Hallows KR, Pastor-Soler NM: PKA regulates vacuolar H⁺-ATPase localization and activity via direct phosphorylation of the a subunit in kidney cells. *J Biol Chem* 285: 24676–24685, 2010
73. Alzamora R, Al-Bataineh MM, Liu W, Gong F, Li H, Thali RF, Joho-Auchli Y, Brunisholz RA, Satlin LM, Neumann D, Hallows KR, Pastor-Soler NM: AMP-activated protein kinase regulates the vacuolar H⁺-ATPase via direct phosphorylation of the A subunit (ATP6V1A) in the kidney. *Am J Physiol Renal Physiol* 305: F943–F956, 2013
74. Gong F, Alzamora R, Smolak C, Li H, Naveed S, Neumann D, Hallows KR, Pastor-Soler NM: Vacuolar H⁺-ATPase apical accumulation in kidney intercalated cells is regulated by PKA and AMP-activated protein kinase. *Am J Physiol Renal Physiol* 298: F1162–F1169, 2010
75. Al-bataineh MM, Gong F, Marciszyn AL, Myerburg MM, Pastor-Soler NM: Regulation of proximal tubule vacuolar H⁺-ATPase by PKA and AMP-activated protein kinase. *Am J Physiol Renal Physiol* 306: F981–F995, 2014
76. Hallows KR, Alzamora R, Li H, Gong F, Smolak C, Neumann D, Pastor-Soler NM: AMP-activated protein kinase inhibits alkaline pH- and PKA-induced apical vacuolar H⁺-ATPase accumulation in epididymal clear cells. *Am J Physiol Cell Physiol* 296: C672–C681, 2009
77. Gidon A, Al-Bataineh MM, Jean-Alphonse FG, Stevenson HP, Watanabe T, Louet C, Khatri A, Calero G, Pastor-Soler NM, Gardella TJ, Vilardaga JP: Endosomal GPCR signaling turned off by negative feedback actions of PKA and v-ATPase. *Nat Chem Biol* 10: 707–709, 2014
78. Păunescu TG, Ljubojevic M, Russo LM, Winter C, McLaughlin MM, Wagner CA, Breton S, Brown D: cAMP stimulates apical V-ATPase accumulation, microvillar elongation, and proton extrusion in kidney collecting duct A-intercalated cells. *Am J Physiol Renal Physiol* 298: F643–F654, 2010
79. Paunescu TG, Da Silva N, Russo LM, McKee M, Lu HA, Breton S, Brown D: Association of soluble adenylyl cyclase with the V-ATPase in renal epithelial cells. *Am J Physiol Renal Physiol* 294: F130–F138, 2008
80. Xu J, Barone S, Li H, Holiday S, Zahedi K, Soleimani M: Slc26a11, a chloride transporter, localizes with the vacuolar H⁺-ATPase of A-intercalated cells of the kidney. *Kidney Int* 80: 926–937, 2011
81. Fisher KD, Codina J, Petrovic S, DuBose TD Jr: Pyk2 regulates H⁺-ATPase-mediated proton secretion in the outer medullary collecting duct via an ERK1/2 signaling pathway. *Am J Physiol Renal Physiol* 303: F1353–F1362, 2012
82. Royaux IE, Wall SM, Karniski LP, Everett LA, Suzuki K, Knepper MA, Green ED: Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc Natl Acad Sci U S A* 98: 4221–4226, 2001
83. Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxeavanis AD, Sheffield VC, Green ED: Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat Genet* 17: 411–422, 1997
84. Scott DA, Wang R, Kreman TM, Sheffield VC, Karniski LP: The Pendred syndrome gene encodes a chloride-iodide transport protein. *Nat Genet* 21: 440–443, 1999
85. Everett LA, Morsli H, Wu DK, Green ED: Expression pattern of the mouse ortholog of the Pendred's syndrome gene (Pds) suggests a key role for pendrin in the inner ear. *Proc Natl Acad Sci U S A* 96: 9727–9732, 1999
86. Royaux IE, Suzuki K, Mori A, Katoh R, Everett LA, Kohn LD, Green ED: Pendrin, the protein encoded by the Pendred syndrome gene (PDS), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. *Endocrinology* 141: 839–845, 2000
87. Pendred V: Deaf-mutism and goitre. *Lancet* 148: 532, 1896
88. Morgans ME, Trotter WR: Association of congenital deafness with goitre; the nature of the thyroid defect. *Lancet* 1: 607–609, 1958
89. Kim YH, Verlander JW, Matthews SW, Kurtz I, Shin W, Weiner ID, Everett LA, Green ED, Nielsen S, Wall SM: Intercalated cell H⁺/OH⁻ transporter expression is reduced in Slc26a4 null mice. *Am J Physiol Renal Physiol* 289: F1262–F1272, 2005
90. Verlander JW, Kim YH, Shin W, Pham TD, Hassell KA, Beierwaltes WH, Green ED, Everett L, Matthews SW, Wall SM: Dietary Cl⁻ restriction upregulates pendrin expression within the apical plasma membrane of type B intercalated cells. *Am J Physiol Renal Physiol* 291: F833–F839, 2006
91. Wall SM, Kim YH, Stanley L, Glapion DM, Everett LA, Green ED, Verlander JW: NaCl restriction upregulates renal Slc26a4 through subcellular redistribution: Role in Cl⁻ conservation. *Hypertension* 44: 982–987, 2004
92. Sansom SC, Weinman EJ, O'Neil RG: Microelectrode assessment of chloride-conductive properties of cortical collecting duct. *Am J Physiol* 247: F291–F302, 1984
93. Gong Y, Yu M, Yang J, Gonzales E, Perez R, Hou M, Tripathi P, Hering-Smith KS, Hamm LL, Hou J: The Cap1-claudin-4 regulatory pathway is important for renal chloride reabsorption and blood pressure regulation. *Proc Natl Acad Sci U S A* 111: E3766–E3774, 2014
94. Kurtz TW, Al-Bander HA, Morris RC Jr: "Salt-sensitive" essential hypertension in men. Is the sodium ion alone important? *N Engl J Med* 317: 1043–1048, 1987
95. Hafner P, Grimaldi R, Capuano P, Capasso G, Wagner CA: Pendrin in the mouse kidney is primarily regulated by Cl⁻ excretion but also by systemic metabolic acidosis. *Am J Physiol Cell Physiol* 295: C1658–C1667, 2008
96. Pech V, Zheng W, Pham TD, Verlander JW, Wall SM: Angiotensin II activates H⁺-ATPase in type A intercalated cells. *J Am Soc Nephrol* 19: 84–91, 2008
97. Mohebbi N, Perna A, van der Wijst J, Becker HM, Capasso G, Wagner CA: Regulation of two renal chloride transporters, AE1 and pendrin, by electrolytes and aldosterone. *PLoS ONE* 8: e55286, 2013
98. Kim YH, Pech V, Spencer KB, Beierwaltes WH, Everett LA, Green ED, Shin W, Verlander JW, Sutliff RL, Wall SM: Reduced ENaC protein abundance contributes to the lower blood pressure observed in pendrin-null mice. *Am J Physiol Renal Physiol* 293: F1314–F1324, 2007
99. Verlander JW, Hong S, Pech V, Bailey JL, Agazatian D, Matthews SW, Coffman TM, Le T, Inagami T, Whitehill FM, Weiner ID, Farley DB, Kim YH, Wall SM: Angiotensin II acts through the angiotensin 1a receptor to upregulate pendrin. *Am J Physiol Renal Physiol* 301: F1314–F1325, 2011
100. Pech V, Pham TD, Hong S, Weinstein AM, Spencer KB, Duke BJ, Walp E, Kim YH, Sutliff RL, Bao HF, Eaton DC, Wall SM: Pendrin modulates ENaC function by changing luminal HCO₃⁻. *J Am Soc Nephrol* 21: 1928–1941, 2010
101. Wall SM, Hassell KA, Royaux IE, Green ED, Chang JY, Shipley GL, Verlander JW: Localization of pendrin in mouse kidney. *Am J Physiol Renal Physiol* 284: F229–F241, 2003

102. Kim YH, Kwon TH, Frische S, Kim J, Tisher CC, Madsen KM, Nielsen S: Immunocytochemical localization of pendrin in intercalated cell subtypes in rat and mouse kidney. *Am J Physiol Renal Physiol* 283: F744–F754, 2002
103. Scott DA, Karniski LP: Human pendrin expressed in *Xenopus laevis* oocytes mediates chloride/formate exchange. *Am J Physiol Cell Physiol* 278: C207–C211, 2000
104. Azroyan A, Laghmani K, Crambert G, Mordasini D, Doucet A, Edwards A: Regulation of pendrin by pH: dependence on glycosylation. *Biochem J* 434: 61–72, 2011
105. Alesutan I, Daryadel A, Mohebbi N, Pelzl L, Leibrock C, Voelkl J, Bourgeois S, Dossena S, Nofziger C, Paulmichl M, Wagner CA, Lang F: Impact of bicarbonate, ammonium chloride, and acetazolamide on hepatic and renal SLC26A4 expression. *Cell Physiol Biochem* 28: 553–558, 2011
106. Kim YH, Pham YD, Zheng W, Hong S, Baylis C, Pech V, Beierwaltes WH, Farley DB, Braverman LE, Verlander JW, Wall SM: Role of pendrin in iodide balance: going with the flow. *Am J Physiol Renal Physiol* 297: F1069–F1079, 2009
107. Leviel F, Hübner CA, Houillier P, Morla L, El Moghrabi S, Brideau G, Hassan H, Parker MD, Kurth I, Kougoumtzes A, Sinning A, Pech V, Riemondy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, Eladari D: The Na⁺-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na⁺ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest* 120: 1627–1635, 2010
108. Hentschke M, Hentschke S, Borgmeyer U, Hübner CA, Kurth I: The murine AE4 promoter predominantly drives type B intercalated cell specific transcription. *Histochem Cell Biol* 132: 405–412, 2009
109. Purkerson JM, Heintz EV, Nakamori A, Schwartz GJ: Insights into acidosis-induced regulation of SLC26A4 (pendrin) and SLC4A9 (AE4) transporters using three-dimensional morphometric analysis of β -intercalated cells. *Am J Physiol Renal Physiol* 307: F601–F611, 2014
110. Verrey F, Hummler E, Schild L, Rossier B: Control of Na⁺ transport by aldosterone. In: *The Kidney: Physiology and Pathophysiology*, edited by Seldin DW, Giebisch G, Philadelphia, Williams & Wilkins, 2000, pp 1441–1471
111. Karim Z, Szutkowska M, Vernimmen C, Bichara M: Renal handling of NH₃/NH₄⁺: Recent concepts. *Nephron, Physiol* 101: 77–81, 2005
112. Sebastian A, Sutton JM, Hulter HN, Schambelan M, Poler SM: Effect of mineralocorticoid replacement therapy on renal acid-base homeostasis in adrenalectomized patients. *Kidney Int* 18: 762–773, 1980
113. Pearce D, Soundararajan R, Trimpert C, Kashlan OB, Deen PM, Kohan DE: Collecting duct principal cell transport processes and their regulation [published online ahead of print May 29, 2014]. *Clin J Am Soc Nephrol* doi: 10.2215/CJN.05760513
114. Prieto-Carrasquero MC, Botros FT, Kobori H, Navar LG: Collecting duct renin: a major player in angiotensin II-dependent hypertension. *J Am Soc Hypertens* 3: 96–104, 2009
115. Frindt G, Palmer LG: Low-conductance K channels in apical membrane of rat cortical collecting tubule. *Am J Physiol* 256: F143–F151, 1989
116. Schambelan M, Sebastian A, Katuna BA, Arteaga E: Adrenocortical hormone secretory response to chronic NH₄Cl-induced metabolic acidosis. *Am J Physiol* 252: E454–E460, 1987
117. Wagner CA, Devuyst O, Bourgeois S, Mohebbi N: Regulated acid-base transport in the collecting duct. *Pflügers Arch* 458: 137–156, 2009
118. Estilo G, Liu W, Pastor-Soler N, Mitchell P, Carattino MD, Kleyman TR, Satlin LM: Effect of aldosterone on BK channel expression in mammalian cortical collecting duct. *Am J Physiol Renal Physiol* 295: F780–F788, 2008
119. Rothenberger F, Velic A, Stehberger PA, Kovacicova J, Wagner CA: Angiotensin II stimulates vacuolar H⁺-ATPase activity in renal acid-secretory intercalated cells from the outer medullary collecting duct. *J Am Soc Nephrol* 18: 2085–2093, 2007
120. Ackermann D, Gresko N, Carrel M, Löffing-Cueni D, Habermehl D, Gomez-Sanchez C, Rossier BC, Löffing J: In vivo nuclear translocation of mineralocorticoid and glucocorticoid receptors in rat kidney: Differential effect of corticosteroids along the distal tubule. *Am J Physiol Renal Physiol* 299: F1473–F1485, 2010
121. Bostanjoglo M, Reeves WB, Reilly RF, Velázquez H, Robertson N, Litwack G, Morsing P, Dörup J, Bachmann S, Ellison DH: 11 β -hydroxysteroid dehydrogenase, mineralocorticoid receptor, and thiazide-sensitive Na-Cl cotransporter expression by distal tubules. *J Am Soc Nephrol* 9: 1347–1358, 1998
122. Farman N, Rafestin-Oblin ME: Multiple aspects of mineralocorticoid selectivity. *Am J Physiol Renal Physiol* 280: F181–F192, 2001
123. Stanton BA: Regulation by adrenal corticosteroids of sodium and potassium transport in loop of Henle and distal tubule of rat kidney. *J Clin Invest* 78: 1612–1620, 1986
124. Nguyen G, Delarue F, Burcklé C, Bouzahir L, Giller T, Sraer JD: Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 109: 1417–1427, 2002
125. Batenburg WW, Krop M, Garrelds IM, de Vries R, de Bruin RJ, Burcklé CA, Müller DN, Bader M, Nguyen G, Danser AH: Prorenin is the endogenous agonist of the (pro)renin receptor. Binding kinetics of renin and prorenin in rat vascular smooth muscle cells overexpressing the human (pro)renin receptor. *J Hypertens* 25: 2441–2453, 2007
126. Jan Danser AH, Batenburg WW, van Esch JH: Prorenin and the (pro)renin receptor—an update. *Nephrol Dial Transplant* 22: 1288–1292, 2007
127. Ludwig J, Kerscher S, Brandt U, Pfeiffer K, Getlawi F, Apps DK, Schägger H: Identification and characterization of a novel 9.2-kDa membrane sector-associated protein of vacuolar proton-ATPase from chromaffin granules. *J Biol Chem* 273: 10939–10947, 1998
128. Yoshikawa A, Aizaki Y, Kusano K, Kishi F, Susumu T, Iida S, Ishiura S, Nishimura S, Shichiri M, Senbonmatsu T: The (pro)renin receptor is cleaved by ADAM19 in the Golgi leading to its secretion into extracellular space. *Hypertens Res* 34: 599–605, 2011
129. Cousin C, Bracquart D, Contrepas A, Corvol P, Muller L, Nguyen G: Soluble form of the (pro)renin receptor generated by intracellular cleavage by furin is secreted in plasma. *Hypertension* 53: 1077–1082, 2009
130. Ichihara A, Kaneshiro Y, Suzuki F: Prorenin receptor blockers: effects on cardiovascular complications of diabetes and hypertension. *Expert Opin Investig Drugs* 15: 1137–1139, 2006
131. Gonzalez AA, Lara LS, Luffman C, Seth DM, Prieto MC: Soluble form of the (pro)renin receptor is augmented in the collecting duct and urine of chronic angiotensin II-dependent hypertensive rats. *Hypertension* 57: 859–864, 2011
132. Rohrwasser A, Morgan T, Dillon HF, Zhao L, Callaway CW, Hillas E, Zhang S, Cheng T, Inagami T, Ward K, Terreros DA, Lalouel JM: Elements of a paracrine tubular renin-angiotensin system along the entire nephron. *Hypertension* 34: 1265–1274, 1999
133. Advani A, Kelly DJ, Cox AJ, White KE, Advani SL, Thai K, Connelly KA, Yuen D, Trogadis J, Herzenberg AM, Kuliszewski MA, Leong-Poi H, Gilbert RE: The (Pro)renin receptor: site-specific and functional linkage to the vacuolar H⁺-ATPase in the kidney. *Hypertension* 54: 261–269, 2009
134. Ichihara A, Sakoda M, Kurauchi-Mito A, Kaneshiro Y, Itoh H: Renin, prorenin and the kidney: a new chapter in an old saga. *J Nephrol* 22: 306–311, 2009
135. Kaneshiro Y, Ichihara A, Takemitsu T, Sakoda M, Suzuki F, Nakagawa T, Hayashi M, Inagami T: Increased expression of cyclooxygenase-2 in the renal cortex of human prorenin receptor gene-transgenic rats. *Kidney Int* 70: 641–646, 2006
136. Deinum J, Tarnow L, van Gool JM, de Bruin RA, Derckx FH, Schalekamp MA, Parving HH: Plasma renin and prorenin and renin gene variation in patients with insulin-dependent diabetes mellitus and nephropathy. *Nephrol Dial Transplant* 14: 1904–1911, 1999
137. DuBose TD Jr, Goode AK: Isolated renal tubular disorder: Mechanisms and clinical expression. In: *Schrier's Diseases of the Kidney*, edited by Coffman TM, Falk RJ, Molitoris B, Neilson EG, Schrier RW, Philadelphia, Wolters Kluwer Lippincott, Williams & Wilkins, 2007, pp 587–614.

138. Douglas JB, Healy JK: Nephrotoxic effects of amphotericin B, including renal tubular acidosis. *Am J Med* 46: 154–162, 1969
139. Sebastian A, McSherry E, Morris RC Jr: Renal potassium wasting in renal tubular acidosis (RTA): Its occurrence in types 1 and 2 RTA despite sustained correction of systemic acidosis. *J Clin Invest* 50: 667–678, 1971
140. Karet FE, Finberg KE, Nayir A, Bakaloglu A, Ozen S, Hulton SA, Sanjad SA, Al-Sabban EA, Medina JF, Lifton RP: Localization of a gene for autosomal recessive distal renal tubular acidosis with normal hearing (rdRTA2) to 7q33–34. *Am J Hum Genet* 65: 1656–1665, 1999
141. Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, Rodriguez-Soriano J, Santos F, Cremers CW, Di Pietro A, Hoffbrand BI, Winiarski J, Bakaloglu A, Ozen S, Dusunsel R, Goodyer P, Hulton SA, Wu DK, Skvorak AB, Morton CC, Cunningham MJ, Jha V, Lifton RP: Mutations in the gene encoding B1 subunit of H⁺-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* 21: 84–90, 1999
142. Stover EH, Borthwick KJ, Bavalia C, Eady N, Fritz DM, Rungroj N, Giersch AB, Morton CC, Axon PR, Akil I, Al-Sabban EA, Baguley DM, Bianca S, Bakaloglu A, Bircan Z, Chauveau D, Clermont MJ, Guala A, Hulton SA, Kroes H, Li Volti G, Mir S, Mocan H, Nayir A, Ozen S, Rodriguez Soriano J, Sanjad SA, Tasic V, Taylor CM, Topaloglu R, Smith AN, Karet FE: Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. *J Med Genet* 39: 796–803, 2002
143. Karet FE, Gainza FJ, Györy AZ, Unwin RJ, Wrong O, Tanner MJ, Nayir A, Alpay H, Santos F, Hulton SA, Bakaloglu A, Ozen S, Cunningham MJ, di Pietro A, Walker WG, Lifton RP: Mutations in the chloride-bicarbonate exchanger gene AE1 cause autosomal dominant but not autosomal recessive distal renal tubular acidosis. *Proc Natl Acad Sci U S A* 95: 6337–6342, 1998
144. Bruce LJ, Cope DL, Jones GK, Schofield AE, Burley M, Povey S, Unwin RJ, Wrong O, Tanner MJ: Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. *J Clin Invest* 100: 1693–1707, 1997
145. Ribeiro ML, Alloisio N, Almeida H, Gomes C, Texier P, Lemos C, Mimoso G, Morlé L, Bey-Cabet F, Rudigoz RC, Delaunay J, Tamagnini G: Severe hereditary spherocytosis and distal renal tubular acidosis associated with the total absence of band 3. *Blood* 96: 1602–1604, 2000
146. Breton S, Alper SL, Gluck SL, Sly WS, Barker JE, Brown D: Depletion of intercalated cells from collecting ducts of carbonic anhydrase II-deficient (CAR2 null) mice. *Am J Physiol* 269: F761–F774, 1995
147. Sebastian A, McSherry E, Morris RC Jr: Impaired renal conservation of sodium and chloride during sustained correction of systemic acidosis in patients with type 1, classic renal tubular acidosis. *J Clin Invest* 58: 454–469, 1976
148. Finberg KE, Wagner CA, Bailey MA, Paunescu TG, Breton S, Brown D, Giebisch G, Geibel JP, Lifton RP: The B1-subunit of the H(+) ATPase is required for maximal urinary acidification. *Proc Natl Acad Sci U S A* 102: 13616–13621, 2005
149. Guan Y, Zhang Y, Breyer RM, Fowler B, Davis L, Hébert RL, Breyer MD: Prostaglandin E2 inhibits renal collecting duct Na⁺ absorption by activating the EP1 receptor. *J Clin Invest* 102: 194–201, 1998
150. Hébert RL, Jacobson HR, Fredin D, Breyer MD: Evidence that separate PGE2 receptors modulate water and sodium transport in rabbit cortical collecting duct. *Am J Physiol* 265: F643–F650, 1993
151. Ando Y, Asano Y: Luminal prostaglandin E2 modulates sodium and water transport in rabbit cortical collecting ducts. *Am J Physiol* 268: F1093–F1101, 1995
152. Krupin T, Sly WS, Whyte MP, Dodgson SJ: Failure of acetazolamide to decrease intraocular pressure in patients with carbonic anhydrase II deficiency. *Am J Ophthalmol* 99: 396–399, 1985
153. Zietse R, Zoutendijk R, Hoorn EJ: Fluid, electrolyte and acid-base disorders associated with antibiotic therapy. *Nat Rev Nephrol* 5: 193–202, 2009
154. Pertovaara M, Korpela M, Kouri T, Pasternack A: The occurrence of renal involvement in primary Sjögren's syndrome: A study of 78 patients. *Rheumatology (Oxford)* 38: 1113–1120, 1999
155. Ren H, Wang WM, Chen XN, Zhang W, Pan XX, Wang XL, Lin Y, Zhang S, Chen N: Renal involvement and followup of 130 patients with primary Sjögren's syndrome. *J Rheumatol* 35: 278–284, 2008
156. Cohen EP, Bastani B, Cohen MR, Kolner S, Hemken P, Gluck SL: Absence of H(+) -ATPase in cortical collecting tubules of a patient with Sjogren's syndrome and distal renal tubular acidosis. *J Am Soc Nephrol* 3: 264–271, 1992
157. Devuyt O, Lemaire M, Mohebbi N, Wagner CA: Autoantibodies against intercalated cells in Sjögren's syndrome. *Kidney Int* 76: 229, 2009
158. DeFranco PE, Haragsim L, Schmitz PG, Bastani B: Absence of vacuolar H(+) -ATPase pump in the collecting duct of a patient with hypokalemic distal renal tubular acidosis and Sjögren's syndrome. *J Am Soc Nephrol* 6: 295–301, 1995
159. Walsh S, Turner CM, Töy A, Wagner C, Jaeger P, Laing C, Unwin R: Immunohistochemical comparison of a case of inherited distal renal tubular acidosis (with a unique AE1 mutation) with an acquired case secondary to autoimmune disease. *Nephrol Dial Transplant* 22: 807–812, 2007
160. Trepiccione F, Christensen BM: Lithium-induced nephrogenic diabetes insipidus: new clinical and experimental findings. *J Nephrol* 23[Suppl 16]: S43–S48, 2010
161. Grünfeld JP, Rossier BC: Lithium nephrotoxicity revisited. *Nat Rev Nephrol* 5: 270–276, 2009
162. Batlle D, Gaviria M, Grupp M, Arruda JA, Wynn J, Kurtzman NA: Distal nephron function in patients receiving chronic lithium therapy. *Kidney Int* 21: 477–485, 1982
163. Christensen BM, Marples D, Kim YH, Wang W, Frøkiaer J, Nielsen S: Changes in cellular composition of kidney collecting duct cells in rats with lithium-induced NDI. *Am J Physiol Cell Physiol* 286: C952–C964, 2004
164. Kishore BK, Ecelbarger CM: Lithium: A versatile tool for understanding renal physiology. *Am J Physiol Renal Physiol* 304: F1139–F1149, 2013
165. Kuure S, Popsueva A, Jakobson M, Sainio K, Sariola H: Glycogen synthase kinase-3 inactivation and stabilization of beta-catenin induce nephron differentiation in isolated mouse and rat kidney mesenchymes. *J Am Soc Nephrol* 18: 1130–1139, 2007
166. Pulkkinen K, Murugan S, Vainio S: Wnt signaling in kidney development and disease. *Organogenesis* 4: 55–59, 2008
167. Park EY, Kim WY, Kim YM, Lee JH, Han KH, Weiner ID, Kim J: Proposed mechanism in the change of cellular composition in the outer medullary collecting duct during potassium homeostasis. *Histol Histopathol* 27: 1559–1577, 2012
168. Heering P, Ivens K, Aker S, Grabensee B: Distal tubular acidosis induced by FK506. *Clin Transplant* 12: 465–471, 1998
169. Mohebbi N, Mihailova M, Wagner CA: The calcineurin inhibitor FK506 (tacrolimus) is associated with transient metabolic acidosis and altered expression of renal acid-base transport proteins. *Am J Physiol Renal Physiol* 297: F499–F509, 2009
170. Peng H, Vijayakumar S, Schiene-Fischer C, Li H, Purkerson JM, Malesevic M, Liebscher J, Al-Awqati Q, Schwartz GJ: Secreted cyclophilin A, a peptidylprolyl cis-trans isomerase, mediates matrix assembly of hensin, a protein implicated in epithelial differentiation. *J Biol Chem* 284: 6465–6475, 2009
171. Korhonen TK, Virkola R, Holthöfer H: Localization of binding sites for purified Escherichia coli P fimbriae in the human kidney. *Infect Immun* 54: 328–332, 1986
172. Chassin C, Goujon JM, Darche S, du Merle L, Bens M, Cluzeaud F, Werts C, Ogier-Denis E, Le Bouguénec C, Buzoni-Gatel D, Vandewalle A: Renal collecting duct epithelial cells react to pyelonephritis-associated Escherichia coli by activating distinct TLR4-dependent and -independent inflammatory pathways. *J Immunol* 177: 4773–4784, 2006
173. Gluba A, Banach M, Hannam S, Mikhailidis DP, Sakowicz A, Rysz J: The role of Toll-like receptors in renal diseases. *Nat Rev Nephrol* 6: 224–235, 2010

174. Ali AS, Townes CL, Hall J, Pickard RS: Maintaining a sterile urinary tract: The role of antimicrobial peptides. *J Urol* 182: 21–28, 2009
175. Spencer JD, Schwaderer AL, Dirosario JD, McHugh KM, McGillivray G, Justice SS, Carpenter AR, Baker PB, Harder J, Hains DS: Ribonuclease 7 is a potent antimicrobial peptide within the human urinary tract. *Kidney Int* 80: 174–80, 2011.
176. Lehmann J, Retz M, Harder J, Krams M, Kellner U, Hartmann J, Hohgräwe K, Raffenberg U, Gerber M, Loch T, Weichert-Jacobsen K, Stöckle M: Expression of human beta-defensins 1 and 2 in kidneys with chronic bacterial infection. *BMC Infect Dis* 2: 20, 2002
177. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P: Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol* 14: 2534–2543, 2003
178. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, Schmidt-Ott KM, Chen X, Li JY, Weiss S, Mishra J, Cheema FH, Markowitz G, Suganami T, Sawai K, Mukoyama M, Kunis C, D'Agati V, Devarajan P, Barasch J: Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 115: 610–621, 2005
179. Paragas N, Kulkarni R, Werth M, Schmidt-Ott KM, Forster C, Deng R, Zhang Q, Singer E, Klose AD, Shen TH, Francis KP, Ray S, Vijayakumar S, Seward S, Bovino ME, Xu K, Takabe Y, Amaral FE, Mohan S, Wax R, Corbin K, Sanna-Cherchi S, Mori K, Johnson L, Nickolas T, D'Agati V, Lin CS, Qiu A, Al-Awqati Q, Ratner AJ, Barasch J: α -Intercalated cells defend the urinary system from bacterial infection. *J Clin Invest* 124: 2963–2976, 2014
180. Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, Viltard M, Yu W, Forster CS, Gong G, Liu Y, Kulkarni R, Mori K, Kalandadze A, Ratner AJ, Devarajan P, Landry DW, D'Agati V, Lin CS, Barasch J: The Ngal reporter mouse detects the response of the kidney to injury in real time. *Nat Med* 17: 216–222, 2011
181. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK: The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 10: 1033–1043, 2002
182. Barasch J, Mori K: Cell biology: Iron thievery. *Nature* 432: 811–813, 2004
183. Miró-Julià C, Escoda-Ferran C, Carrasco E, Moeller JB, Vadekaer DF, Gao X, Paragas N, Oliver J, Holmskov U, Al-Awqati Q, Lozano F: Expression of the innate defense receptor S5D-SRCRB in the urogenital tract. *Tissue Antigens* 83: 273–285, 2014
184. Pastor-Soler NM, Hallows KR, Smolak C, Gong F, Brown D, Breton S: Alkaline pH- and cAMP-induced V-ATPase membrane accumulation is mediated by protein kinase A in epididymal clear cells. *Am J Physiol Cell Physiol* 294: C488–C494, 2008

Published online ahead of print. Publication date available at www.cjasn.org.



Control of Urinary Drainage and Voiding

Warren G. Hill

Abstract

Urine differs greatly in ion and solute composition from plasma and contains harmful and noxious substances that must be stored for hours and then eliminated when it is socially convenient to do so. The urinary tract that handles this output is composed of a series of pressurizable muscular compartments separated by sphincteric structures. With neural input, these structures coordinate the delivery, collection, and, ultimately, expulsion of urine. Despite large osmotic and chemical gradients in this waste fluid, the bladder maintains a highly impermeable surface in the face of a physically demanding biomechanical environment, which mandates recurring cycles of surface area expansion and increased wall tension during filling, followed by rapid wall compression during voiding. Afferent neuronal inflow from mucosa and submucosa communicates sensory information about bladder fullness, and voiding is initiated consciously through coordinated central and spinal efferent outflow to the detrusor, trigonal internal sphincter, and external urethral sphincter after periods of relative quiescence. Provocative new findings suggest that in some cases, lower urinary tract symptoms, such as incontinence, urgency, frequency, overactivity, and pain may be viewed as a consequence of urothelial defects (either urothelial barrier breakdown or inappropriate signaling from urothelial cells to underlying sensory afferents and potentially interstitial cells). This review describes the physiologic and anatomic mechanisms by which urine is moved from the kidney to the bladder, stored, and then released. Relevant clinical examples of urinary tract dysfunction are also discussed.

Clin J Am Soc Nephrol 10: 480–492, 2015. doi: 10.2215/CJN.04520413

Laboratory of Voiding Dysfunction,
Department of
Medicine, Beth Israel
Deaconess Medical
Center and Harvard
Medical School,
Boston, Massachusetts

Correspondence:
Dr. Warren G. Hill,
Laboratory of Voiding
Dysfunction, Beth
Israel Deaconess
Medical Center,
RN349 99 Brookline
Avenue, Boston, MA
02215. Email: whill@
bidmc.harvard.edu

Anatomy of the Lower Urinary Tract

Urinary drainage from the kidneys occurs through the ureters (25–30 cm long in adults), which enter the bladder distally *via* a specialized region near the base of the bladder called the trigone. Unidirectional flow from the kidney pelvis into the ureter and from the ureter to the bladder is assisted by two constrictions at opposite ends: the ureteropelvic junction (UPJ) and the ureterovesical junction (UVJ), respectively. These unique fibromuscular structures, which are not strictly classic sphincters, provide antireflux protection in order to ensure that urine transport occurs in only one direction. The ureters consist of stratified layers composed of epithelium (the urothelium), lamina propria, and smooth muscle.

Ureteral smooth muscle cells are arranged in longitudinal, circular, and spiral bundles to facilitate peristaltic movement of urine toward the bladder. As urine is forced past the UPJ by calyceal and renal pelvis smooth muscular contraction, it enters the ureters as a bolus that travels down to the UVJ and is then extruded into the bladder. Distally, the ureters insert into the bladder at an oblique angle and traverse the muscle over a distance of approximately 1.5 cm. Beginning above the entry point to the detrusor, the ureter is sheathed by a layer of longitudinal smooth muscle. This sheath passes through the vesical wall and then diverges to merge with the deep trigone (1). The intravesical ureter forms a valve, which is important in the prevention of reflux. It also protects the kidney from retrograde exposure to the high pressures generated

by the bladder at voiding and also from infections localized in the bladder. The physical relationship of the UVJ in terms of its length, diameter, and positioning relative to the trigone prevents retrograde flow. Damage to the trigone, congenital abnormalities, or trigonal muscular weakness are all primary causes of vesicoureteral reflux. The complexity of the UVJ makes it the most difficult anastomosis to perform in renal transplantation (2).

The bladder is a highly deformable muscular sac that has two primary functions: storage and expulsion. It features a layered structure similar to that of the ureters, with a highly impermeable urothelium, an intermediate vascularized lamina propria composed of connective tissue, several fibroblastic cell types, and a thick smooth muscle coat called the detrusor. Figure 1 illustrates the laminar nature of these layers and the surface topography of the bladder, which is highly folded and ridged when not fully stretched.

The urethra begins at the lower apex of the bladder neck and is formed of several layers of muscle. Figure 2 shows the anatomy of male and female urethrae. The urethra, on average, is 3.5–5 cm long in women, whereas it is approximately 20 cm long in men. The urethral tube is formed from an inner longitudinal smooth muscle, which, in turn, is surrounded by a thinner circular smooth muscle layer. Urinary continence is maintained in both sexes by an involuntary internal sphincter and a voluntary external urethral sphincter (EUS), sometimes called the rhabdosphincter.

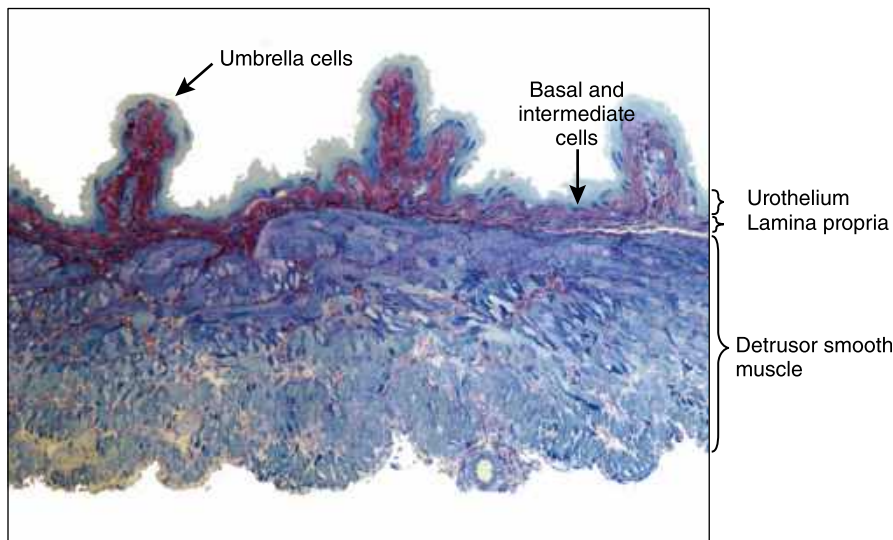


Figure 1. | Mouse bladder sectioned and counterstained with toluidine blue. The layers of the bladder and their relative dimensions are easily visualized by different degrees of red/blue coloration.

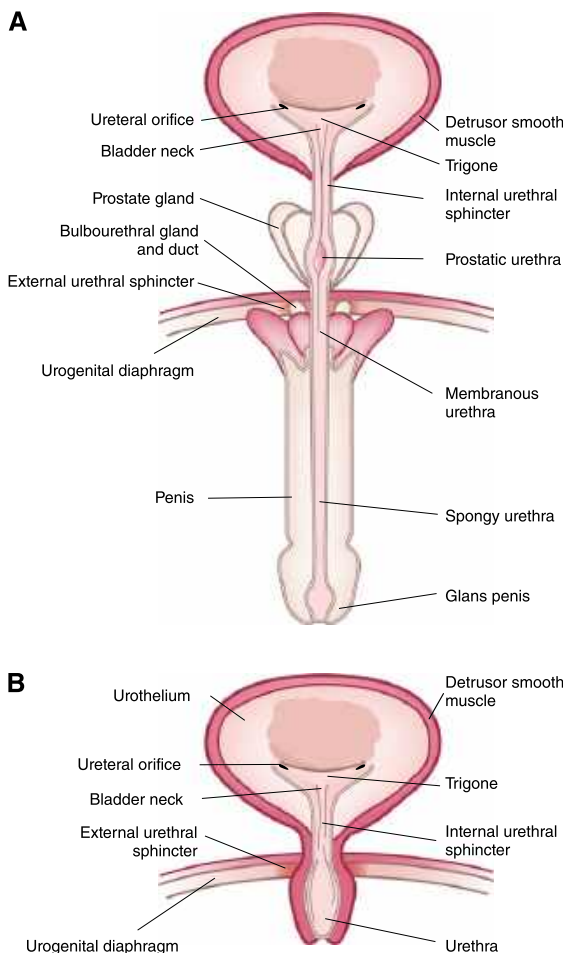


Figure 2. | Lower urinary tract anatomy including bladder and urethra. (A) Human male and (B) female.

Physiology of the Ureters

Urine flow through the ureters occurs by peristalsis, which facilitates unidirectional flow. At normal urine production rates, contraction of the renal pelvis forces a bolus of urine into the ureter, upon which waves of contraction (20–80 cmH₂O; 2–6 times/min) occur behind the bolus and force it distally into relaxed sections with baseline pressures of only 0–5 cmH₂O. When urine production rates become particularly high, boluses can become larger and then merge and may ultimately become essentially a column of fluid. Upon expulsion of urine through the UVJ, the peristaltic wave dissipates.

Ureteral obstruction raises the intraluminal pressure above the obstruction and is usually accompanied by an increase in peristaltic frequency as well as changes in ureteral dimensions. In response to this distal pressure, the ureter becomes dilated and increases in length due to the retention of urine and tissue stretching. If the obstruction is not cleared, the contractions diminish, intraluminal pressures will subsequently diminish to almost baseline levels, and the ureter can ultimately decompensate, losing the ability to contract even if the obstruction is removed. Hence, the early response to calculi is an increase in peristaltic pressure, resulting in increased proximal pressure on the stone and simultaneous relaxation at, and distal to, the blockage. In tandem, these responses are designed to aid in stone passage. However, if this does not prove successful and blockage is maintained, irreversible damage can occur.

Smooth muscle contractions occur in response to increases in intracellular calcium (Ca²⁺); conversely, relaxation occurs when Ca²⁺ drops. This has been exploited therapeutically *via* the use of calcium channel blockers (*e.g.*, nifedipine), which augment relaxation and thereby assist with stone passage, in an approach known as medical expulsive therapy (3,4). Smooth muscle contracts either as a response to neurotransmitters released from firing of motor neurons (neurogenic contractility) or as a result of local intrinsic network signaling originating within the muscle (myogenic or spontaneous contractility).

Myogenic Contractility

Electrical activity within the pyeloureters causes smooth muscle contractility at the kidney pelvis and results in coordinated peristalsis. This activity originates in a pacemaker cell network composed of atypical smooth muscle cells (SMCs) and interstitial cells of Cajal-like (ICC-like) cells. These pacemaker cells spontaneously generate electrical activity. Although the precise contribution of these two cell types is not yet defined in ureters, atypical SMCs appear to be the primary pacemakers at the UPJ (5–9). These cells exhibit spontaneous high-frequency Ca^{2+} spikes released from internal stores that propagate through gap junctions to induce transient membrane depolarization in typical SMCs. Depolarization activates an influx of Ca^{2+} through voltage-gated L-type calcium channels on the plasma membrane, which then initiates tonic cytosolic Ca^{2+} oscillations resulting in SMC contraction (10). The role of ICC-like cells is not clear because they form a separate, but interacting, electrically active network, which may be important in peristaltic contraction in distal regions of the ureter (8). Loss of ICC-like cells has been noted in studies of congenital UPJ obstruction (11–14). It is not clear, however, whether this reduction in cell number is a cause of the obstruction or a secondary consequence.

Neurogenic Contractility

Ureters are innervated directly by afferent and efferent fibers. However, peristalsis persists in the presence of tetrodotoxin, which blocks voltage-gated sodium channels and, hence, action potentials. Furthermore, ureters transplanted along with transplant kidneys, which lose their innervation, continue to exhibit peristalsis (15). This strongly suggests that neurogenic regulation is likely to be modulatory in nature (16). Neurotransmitter signaling pathways from both sympathetic and parasympathetic systems include adrenergic (adrenaline/noradrenaline), cholinergic (acetylcholine), and so-called nonadrenergic, noncholinergic effectors, such as ATP.

The sympathetic nervous system plays a prominent role in ureteral contractile function and both α - and β -adrenergic receptors (ARs) are present in human ureters with α -receptors presenting at higher density (17). α -ARs augment contractions and the clinically used α_1 -AR blocker tamsulosin can reduce or block human ureteric contraction (18). Likewise, the α_1 -AR blocker doxazosin also inhibits spontaneous contractions in human ureteral strips and both drugs have found clinical use in assisting ureteral stone passage through the relative relaxant effects (19,20). By contrast, β -ARs enhance ureteral wall relaxation. The β_2 and β_3 subtypes appear to be the predominant receptors mediating relaxation (21,22).

Bladder Accommodation and Expulsion of Urine

The bladder accommodates the flow of urine by maintaining a low intravesical pressure during filling. This compliance in the bladder wall allows volume accommodation and storage, but also ensures that the ureters are not forced to transport urine into a pressurized compartment, with the attendant risk of retrograde flow. Upon voiding, the bladder rapidly contracts and, in a few seconds, develops intravesical pressures of 50–60 cmH_2O before voluntary

sphincter opening allows expulsion of urine at flow rates of 20–30 ml/s. Both accommodation and expulsion of urine require coordinated communication between urothelium, afferent nerves, spinal and hypothalamic centers, efferent pathways, and detrusor and sphincter muscles.

Neural Pathways

During infancy, and in patients who have suffered spinal cord injury, the bladder is capable of reflex emptying, but micturition is not under conscious control. The development of voiding control requires that this immature reflex contraction is inhibited. For infants, peripheral nervous system connections are ready for use at birth, but the coordination that needs to occur between the bladder and sphincter is thought to require maturation of central neural control. The three sets of peripheral nerves involved in bladder accommodation to filling are shown in Figure 3A and synapse onto different regions of the bladder and outlet. During filling, there is low-level activity from bladder afferent fibers that signal *via* the pelvic nerve and this, in turn, stimulates sympathetic outflow to the bladder neck and wall through the hypogastric nerve (Figure 3A). These fibers coordinately regulate negative innervation to the detrusor *via* β_3 -ARs and positive signals to α_1 -ARs in the internal smooth muscle sphincter (23) (Figure 4). There is also pudendal outflow, which keeps the EUS closed. These spinal reflex pathways coordinate relaxation of the detrusor and simultaneous contraction at the internal sphincter and EUS. During accommodation, neurons within the pontine storage center (also known as Barrington's nucleus) in the brain are quiescent.

As the bladder becomes full, afferent firing increases and activates spinobulbospinal reflex pathways. At a critical level of bladder distention, the afferent activity arising from mechanoreceptors in the bladder wall switches the pontine micturition center (PMC) "on" and enhances its activity (Figure 3B). At this point, the accommodation center shuts "off" and ascending afferent input passes through the periaqueductal gray (PAG) relay center before reaching the PMC and elicits efferent outflow (24). Efferent innervation is supplied by the three major nerves shown in Figures 3B and 4, which are the pelvic, hypogastric, and pudendal nerves. The pelvic nerve emerges from sacral region S2–S4 of the spinal column and pelvic ganglion and provides positive (contractile) parasympathetic innervation to the detrusor smooth muscle and negative (or relaxative) innervation to the bladder neck by release of nitric oxide (NO) (Figures 3B and 4). The hypogastric nerve emerges from the inferior mesenteric ganglion after originating in the T11–L2 thoracolumbar segments and these sympathetic fibers are inhibited during micturition.

Somatic (conscious) innervation of the striated EUS is supplied by the pudendal nerve from the S2–S4 sacral region of the spinal column (25). When the bladder is filling, these muscles are under steady constant firing to maintain contraction (Figure 3A), but are inhibited by efferent signaling from the PAG during voiding. Striated muscle is composed of both fast twitch and slow twitch fibers and it is thought that slow twitch fibers that are slower to fatigue are likely responsible for maintenance of tone and the resting urethral pressure profile. The fast twitch fibers are available to come into play during transient events of increased abdominal

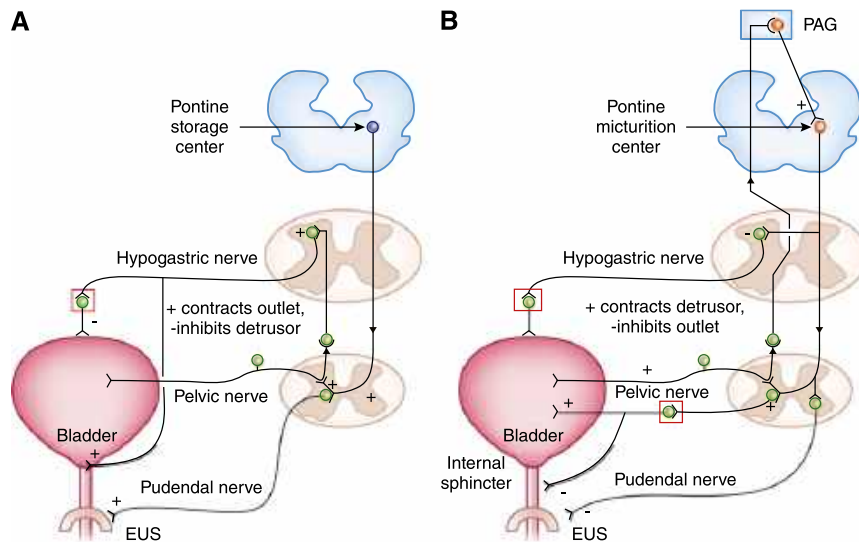


Figure 3. | Neural regulation of the storage/accommodation phase and the voiding phase. (A) Storage reflexes. During filling, there is low-level activity from bladder afferent fibers signaling distension via the pelvic nerve, which in turn stimulates sympathetic outflow to the bladder neck and wall via the hypogastric nerve. This sympathetic stimulation relaxes the detrusor and contracts the bladder neck at the internal sphincter. Afferent pelvic nerve impulses also stimulate the pudendal (somatic) outflow to the external sphincter causing contraction and maintenance of continence. (B) Voiding reflexes. Upon initiation of micturition, there is high-intensity afferent activity signaling wall tension, which activates the brainstem pontine micturition center. Spinobulbospinal reflex can be seen as an ascending signal from afferent pelvic nerve stimulation (left side), which passes through the periaqueductal gray matter before reaching the pontine micturition center and descending (right side) to elicit parasympathetic contraction of the detrusor, and somatic relaxation via the pudendal nerve. EUS, external urethral sphincter; PAG, periaqueductal gray matter. Modified from reference 102 as follows: 1) changes to the color and shape of spinal cord elements; 2) pontine storage center and pontine micturition center colored differently to indicate slightly different neuronal populations; 3) (A) new neural connection between the hypogastric nerve and the bladder outlet, with + and – signs to indicate contractile or relaxative signals, respectively; and (B) several new + and – signs, which were not included in the original.

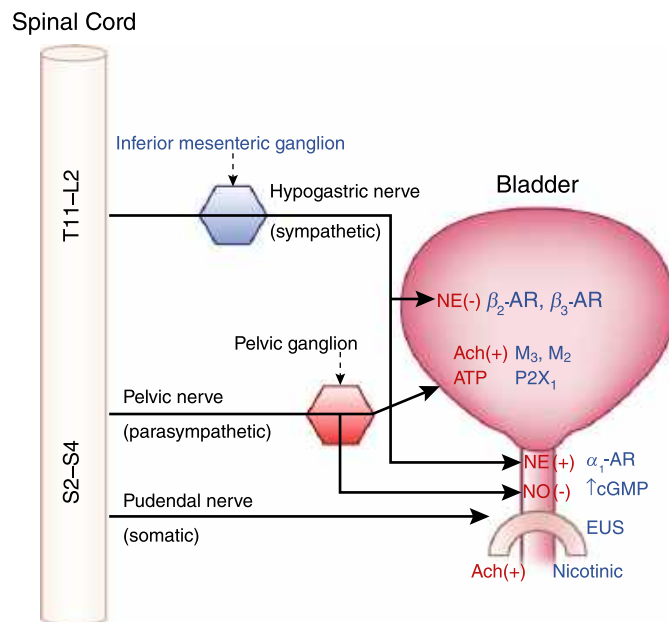


Figure 4. | Major efferent (motor) pathways of the bladder, neurotransmitters (red), receptors (blue), and sites of action. (+), contraction; (–), relaxation; Ach, acetylcholine; NE, norepinephrine; NO, nitric oxide; P2X₁, purinergic receptor X1.

pressure, such as during a cough. Any activities that cause abdominal compression, such as coughing or exercise, produce dramatic increases in the number of units firing with subsequent release of acetylcholine (26) (Figure 4).

Activation of the voiding reflex is under strict voluntary control in continent individuals and it requires complex integration of afferent signals along with conscious perceptions of how full one's bladder is, combined with an

appreciation of the social environment of the moment. The PAG is a key organizing center for several higher brain regions involved in micturition, including the prefrontal cortex with which it has strong connections. Functional imaging studies indicate that the frontal lobes are important in determining the appropriateness of behavioral reactions and cognitive responses to social situations. They receive and pass on sensory signals to areas involved with conscious perception. Furthermore, they communicate in reverse the results of that perception *via* the PAG directly as primary input to the PMC (27). Emphasizing the role of this cortical activity, patients with poor bladder control have been shown to exhibit weak prefrontal activity in response to bladder filling (28). Thus, in healthy people, signaling the need to “hang on,” if need be, arises from decisions about the appropriateness of the timing and results in neural suppression signals arriving at the PMC. The micturition switch is thereby maintained in the off position. Inputs at the PAG arrive from a number of connecting brain regions, including the thalamus, the insula, and the anterior cingulate cortex. In summary, they likely dictate how much attention is paid to bladder filling and what needs to be done about it.

Urothelium

The urothelium provides a continuous superficial lining that stretches from the edge of the renal pelvis to the end of the urethra and is formed of several cell layers that differ in their degree of differentiation. Undifferentiated basal cells line the basement membrane and were thought to replicate to permit the replacement of lost surface cells. However, a recent fate mapping study showed that intermediate cells, which are also relatively undifferentiated, contain the progenitor population for repopulating surface umbrella cells (29). In the relaxed bladder, the epithelial layers can be 4–8 cells deep; however, when the urothelium is distended, it becomes much thinner with fewer apparent cells in the cross-section. The umbrella cells sit on top and interface with the urine as a single layer of flagstone-shaped cells that have tight junctions and a highly impermeable apical plasma membrane. Because water, acid (H^+), ammonia, urea, and carbon dioxide are normally highly permeable across biologic membranes, the apical membrane of the umbrella cell has a unique lipid and protein composition that makes it the least permeable epithelium in the human body (30–33).

Breakdown of the epithelial barrier can occur in a number of clinical settings including infection by uropathogenic *Escherichia coli*, radiation injury, carcinoma, and Hunner’s ulcers that are sometimes seen in interstitial cystitis. Interstitial cystitis, or painful bladder syndrome as it is now named, is a complex disease of undefined etiology, characterized by chronic lower urinary tract and/or pelvic pain that may be accompanied by dysuria, urgency, and frequency. Diagnosis is based on exclusionary criteria, such as absence of infection and carcinoma. Although recent research has emphasized the role of extrabladder factors (*e.g.*, neurogenic) in pathogenesis and potential therapy, there remains a body of evidence implicating dysfunction of the urothelium in the course and severity of the disease. Plausible models in some patients at least, and in cats (34), suggest that damage to the urothelium, which is not balanced by successful repair, causes ongoing symptomatology.

In addition to the passive protective functions that relate to structure, there is now recognition that the urothelium has sensory activity and communicates with other cell types to influence voiding. This conclusion, until recently, has relied on indirect evidence. For example, in response to physical and chemical stimuli, the urothelium releases potent mediators, including the neurotransmitters ATP, acetylcholine, and NO. These are thought to communicate information about the state of tissue stretch and aspects of the external environment to afferent neurons (35,36). An illustration of some of the mechanosensitive pathways activated by stretch is shown in Figure 5. In addition to autocrine effects, sensory afferents extending into and between cells of the urothelium are likely influenced by neurotransmitters released from urothelium.

The urothelium experiences biomechanical forces and appears capable of sensing and responding to them. As the urine accumulates, the three-dimensional conformation of the urothelium changes from highly folded and involuted to smooth and spherical and is subject to mechanical tension that relates to the radius, wall thickness, and intravesical pressure according to LaPlace’s law (Figure 6). Whereas pressure is constant at all points within the bladder, the tension experienced at the different points in the bladder wall varies and is proportional to the radius of curvature (r) divided by the wall thickness. Wall tension, rather than hydrostatic pressure, is the relevant physical parameter that initiates mechanotransduction and, hence, activation of bladder afferents. In hypertrophic small capacity bladders, elevated wall tension occurs at much lower fill volumes and can induce ischemia and vesicoureteral reflux. Any remodeling of the bladder wall that occurs in response to outlet obstruction or spinal cord injury (denervation), for example, results in increased collagen content and a reduction in compliance (37,38). Wall tension increases in such clinical settings leading to altered mechanosensory signaling and a lower volume threshold for the onset of feelings of urgency.

The urothelium secretes ATP (39,40) and other macromolecules as wall tension increases, and high ATP concentrations contribute to nociception by binding purinergic receptor X2/3 ($P2X_{2/3}$) on high threshold urothelial pain afferents. Thus, a question of considerable interest is whether the urothelium, through its ability to sense bladder wall stretch, is capable of regulating voiding and, if so, is it implicated in bladder dysfunction? As a way of addressing this, investigators have recently turned to conditional gene targeting approaches in mice that allow the expression or deletion of genes in specific cell types. Schnegelsberg *et al.* specifically overexpressed nerve growth factor in the urothelium of engineered mice (41). Mice producing excess urothelial nerve growth factor were shown to have nerve fiber hyperplasia, reduced bladder capacity, and increased nonvoiding bladder contractions, indicating that aberrant signaling originating in the urothelium can cause voiding dysfunction (41).

Our laboratory used the uroplakin promotor in an engineered mouse to direct expression of Cre recombinase purely in the urothelium. When this strain is crossed with a mouse that has loxP gene sequences added within or surrounding a gene of interest, the Cre recombinase will excise the DNA between the sites and recombine the ends.

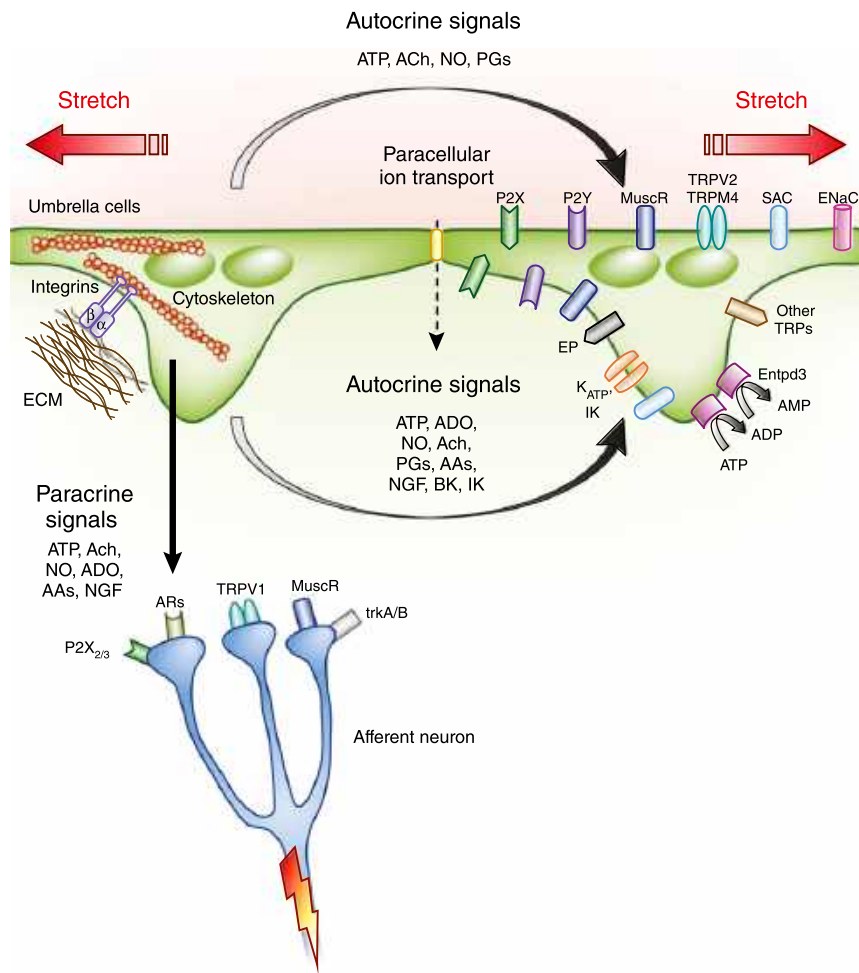


Figure 5. | Mechanically induced signaling from the urothelium in response to membrane stretch. Release of a number of potent mediators occurs in response to urothelial stretch eliciting both autocrine- and paracrine-mediated effects. AA, adrenergic agonist; ADO, adenosine; AR, adrenergic receptor; BK, maxi K channel; EMC, extracellular matrix; ENaC, epithelial sodium channel; Entpd3, ectonucleoside triphosphate diphosphohydrolase 3; EP, PG receptor; NGF, nerve growth factor; P2Y, purinergic receptor Y; SAC, stretch-activated ion channel; trkA/B, tyrosine kinase receptors for nerve growth factor; TRP, transient receptor potential channel.

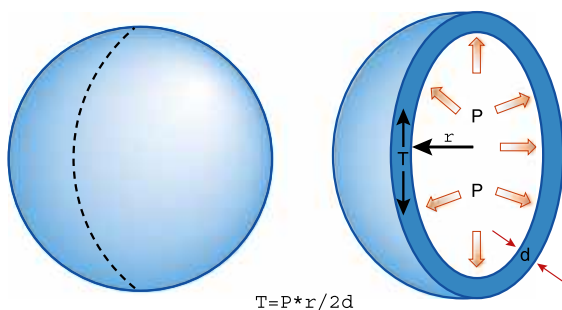


Figure 6. | Illustration of LaPlace's law relating wall tension to pressure, radius, and wall thickness. d, wall thickness; P, pressure; r, radius; T, tension.

This strategy permits cell-specific gene deletion. Using this approach, we deleted β_1 -integrin from the urothelium in mice (42). Integrins connect the intracellular cytoskeleton with the extracellular matrix (see Figure 5) and our

experiments revealed that mice lacking these cell surface receptors in the urothelium were incontinent and exhibited symptoms of overactive bladder (OAB) (42). Because integrins provide important molecular junctions for the transfer of information related to biomechanical stresses, these experiments provided direct evidence that the urothelium regulates voiding through its sensory and signaling functions and may prove to be important in certain bladder disorders.

Detrusor Smooth Muscle

Detrusor contraction is primarily regulated by lumbosacral parasympathetic efferents (Figure 4) that release acetylcholine and ATP to initiate bladder contraction. This can be convincingly demonstrated in bladder strips (without urothelium) mounted in organ baths on isometric tension transducers. Figure 7 shows bladder muscle strips from mice contracting with successively greater force at higher electrical field frequencies due to neurotransmitter release. Inhibition of muscarinic and purinergic signaling with atropine and then α, β -methyl-ATP treatment reduces

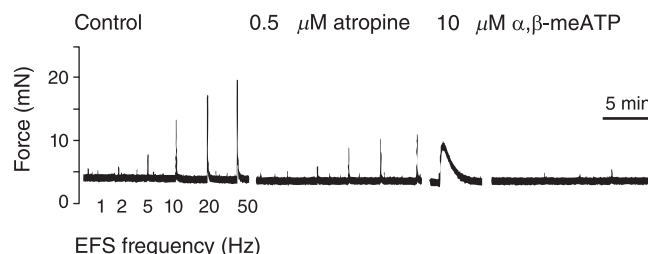


Figure 7. | Electrical field stimulation of mouse bladder muscle strips showing relative contribution of muscarinic and purinergic receptors to contraction force. Muscle strips were prepared by removing urothelium before mounting in organ baths. Increasing the frequency of electrical stimulation increases the muscle contractile force. Addition of atropine, a muscarinic receptor antagonist, diminishes force generation. The force can be further reduced by a purinergic receptor antagonist, α,β -methyl-ATP. Inhibition follows a transient stimulation and is the result of receptor desensitization. EFS, electrical field stimulation.

electrical field-stimulated contractions by approximately 90%, confirming the primacy of acetylcholine and ATP signals (43).

Bladder smooth muscle (BSM) is an excitable tissue, which means that it responds to electrical, chemical, or physical stimuli by a rapid depolarization of the membrane, creating an action potential that can be transmitted to other cells. Action potentials propagate from myocyte to myocyte through gap junctions and result in large-scale contraction. An action potential is the first step in a chain of events after muscarinic and/or purinergic stimulation of receptors, which result in raised intracellular Ca^{2+} . Figure 8 shows several of the major receptor pathways activated in response to neurotransmitter release. The primary receptors for acetylcholine and ATP in BSM are the M_2 , M_3 , and P2X_1 receptors. As the membrane depolarizes as a result of cation influx, subsequent activation of voltage-dependent, L-type Ca^{2+} channels leads to further Ca^{2+} influx and generation of an action potential that can propagate to other myocytes. High intracellular Ca^{2+} results in formation of calcium-calmodulin complexes, which, in turn, activate the myosin light chain kinase and lead to myosin phosphorylation, actomyosin crossbridge formation, and muscle cell contraction (44).

Muscarinic Receptors. M_3 receptors are the primary contractile effector in detrusor, but M_2 receptors are present at 3-fold higher expression density (45). M_2 receptors appear to play a more subtle role in ‘recontracting’ BSM after relaxation (46,47). In the normal bladder, cholinergic contractility is dominant compared with purinergic pathways, and these receptors provide the mainstay target for therapies aimed at symptoms of urgency, incontinence, and OAB. Current anticholinergic drugs approved for OAB include oxybutynin, tolterodine, darifenacin, solifenacin, and trospium. All aim to dampen excess electrical activity leading to inappropriate detrusor contractility; however, their efficacy is questionable, and tolerability is poor because of the anticholinergic side effects.

Purinergic Receptors. Purinergic receptors respond to nucleotides and nucleosides, like ATP, ADP, UTP, UDP, and adenosine. The primary purinergic receptor present on BSM is P2X ; this has been confirmed pharmacologically by testing muscle strips for contractile responses with specific P2X_1 agonists and antagonists by immunostaining (48) and from studies of P2X_1 knockout mice (49). Recent

work from our lab has confirmed the first functional evidence for a purinergic receptor Y (P2Y) in BSM- P2Y_6 (50). P2Y_6 appears to play a role in maintaining spontaneous bladder tone and, in doing so, facilitates the strength of P2X_1 -mediated contractions by almost 50%. This receptor is specifically activated by UDP. Ectonucleotidase, *Entpd1* is present on BSM cell membranes and can degrade both ATP and UTP to ADP/UDP and, ultimately, to the monophosphorylated forms (51). Interestingly, BSM has all the enzymes required to convert ATP to adenosine, which is likely to be important because adenosine causes smooth muscle to relax. In this context, a number of studies have shown that caffeine can exacerbate lower urinary tract symptoms, including OAB (52–54). Although the mechanism remains unclear because caffeine has several cellular effects, it is known to inhibit adenosine receptors at the low doses found from drinking coffee and, thus, may inhibit detrusor relaxation (53). Intriguingly, purinergic signaling becomes more prominent in disease and in aging (55–57) and may emerge to play a dominant role in patients with partial outlet obstruction and interstitial cystitis, potentially accounting for up to 65% of the total contraction force (58).

ARs. ARs in the bladder mediate both contractility and relaxation in response to sympathetic innervation, which primarily occurs at the bladder neck and urethral outlet (Figure 4). The major receptor isoforms present in the human bladder are α_1 and β_3 ; however, expression of α -isoforms as determined at the mRNA and protein levels is very low compared with β -receptors (23). The direct effects of α_1 -AR stimulation on smooth muscle contraction are rather weak, amounting to approximately 10%–40% of the contractile force that results from muscarinic stimulation or KCl depolarization. α_1 -Receptors, therefore, appear to play only a minor functional role in the detrusor, but feature prominently near the bladder outlet in terms of maintaining outlet resistance (59).

The β_3 -receptor is the predominant isoform in the human bladder and accounts for >95% of β -receptors. NE is released from the hypogastric nerves to activate these receptors (Figure 4). The canonical signaling pathway for β -ARs is through elevation of cAMP and generally results in smooth muscle relaxation. It appears likely that activation of the β_3 -receptor may involve interactions with other cellular signaling proteins, such as K channels (60). There

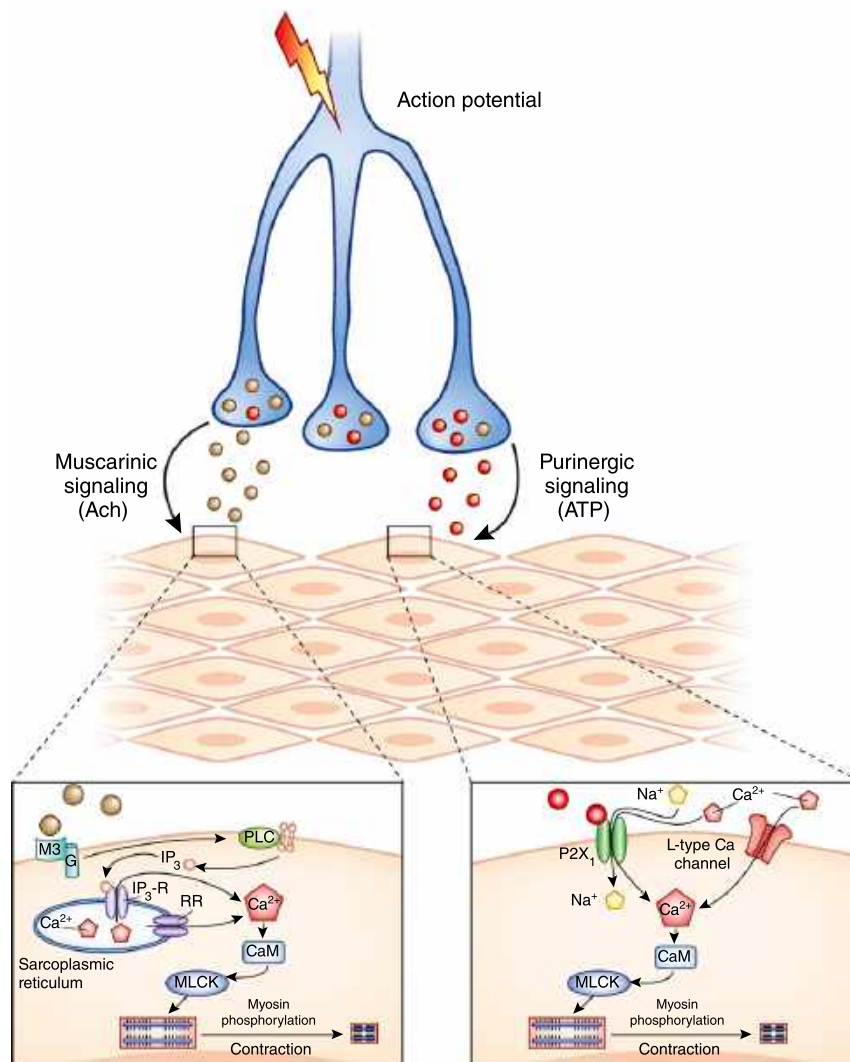


Figure 8. | Molecular mechanisms leading to bladder smooth muscle contraction. Parasympathetic neurons release primary neurotransmitters acetylcholine and ATP, which bind to the M₃ muscarinic receptor and P₂X₁, respectively. This leads to several downstream effects, including membrane depolarization and increased cytosolic Ca²⁺ and ultimately myosin phosphorylation and myocyte contraction. CaM, calmodulin; G, G protein; IP₃, inositol trisphosphate; IP₃-R, inositol trisphosphate receptor; MLCK, myosin light chain kinase; PLC, phospholipase C; RR, ryanodine receptor.

appears to be good evidence for both cAMP-dependent, as well as independent, effects (61). Mirabegron (sold as Myrbetriq) is a β_3 -receptor agonist recently approved as a first-in-class agent designed to treat OAB, urgency, and leakage by promoting relaxation of BSM.

K Channels. BSM expresses a rich variety of K channels, including large-conductance calcium-activated K (BK or maxi K), ATP-sensitive K (K_{ATP}), small-conductance K (SK), inwardly rectifying K (K_{ir}), and voltage-activated K (K_v) channels. As noted above, the effects of β -AR stimulation appear to be at least partially mediated by activation of BK channels in rat, guinea pig, and human bladder (62–64). Overall, it appears that pharmacologic activation of virtually all K channels leads to smooth muscle relaxation as a result of K efflux hyperpolarizing the membrane potential (65–68).

NO. In addition to the primary sympathetic and parasympathetic neurotransmitters, afferent and efferent nerves are known to secrete NO. The expression of NO synthase-containing nerves is highest in the outlet region of the bladder (69,70). Neuronal release of NO primarily elicits relaxation responses at the level of the neck and urethra to facilitate micturition (71). In the cytoplasm, it activates guanylate cyclase and thus increases cGMP, which then activates protein kinase G. In the clinical setting, this pathway has been targeted through use of phosphodiesterase inhibitors, such as sildenafil, which result in elevated levels of intracellular cGMP and enhanced relaxation of bladder neck and urethra (72). In one study, a group of healthy male volunteers, given a NO donor sublingually and studied urodynamically, exhibited lowered bladder outlet resistance characterized by reduced detrusor pressure during voiding and a lower maximal flow rate (73).

Bladder ICC: An Old Cell Newly Implicated in Lower Urinary Tract Symptoms

Embedded deep within the lamina propria and intercalated within the detrusor smooth muscle surrounding muscle bundles are a unique population of cells with stellate morphology. In the last decade, these have been identified as bladder ICCs (BICCs) and the emerging consensus is that they are likely to play an important role in modulating bladder motility (74–81). ICCs were first identified in the gastrointestinal tract where they function as pacemakers, neuron transducers, and mechanosensors to modulate smooth muscle motility (82–85), and more than a dozen gut disorders are linked to ICC dysfunction, including gastroparesis and intestinal pseudo-obstruction (86,87). In urinary bladder, much less is understood. We and others recently demonstrated that ICC markers are expressed in BICCs, including c-kit, anoctamin 1, gap junction protein connexin 43, and CD34 (88). These cells are closely associated with intramural nerves and BSM cells (89–91). The overlying schematic in Figure 9 illustrates the position they occupy in the bladder wall. Figure 9 also shows confocal images of BICCs with the top panels showing stellate-like morphology for cells staining positive with antibodies for c-kit (Figure 9A) and for the ATP breakdown enzyme NTPDase 2 (Figure 9B). These cells occur deep in BSM; Figure 9F shows that they are intimately associated with BSM (labeled with α -smooth muscle actin). BICCs are implicated in bladder diseases, such as megacystis microcolon intestinal hypoperistalsis syndrome, a lethal congenital disease in which infants have no ICCs (92,93). In patients with diabetes, decreased BICCs were observed and this was suggested to contribute to incontinence (74). Alterations to BICC function have also been reported in OAB and obstructed bladder in both humans and animal models (79,92,94–97). Increased expression of connexin 43 has been reported in patients with OAB, suggesting increased signal transduction between BICCs and BSM cells, potentially leading to motility problems (98,99). This cell is just beginning to attract attention as a possible contributor to bladder motility disorders but it is not currently known whether alterations in BICCs are primary or secondary to disease processes.

Urethral Contractile Physiology

The urethra is a distensible muscular structure composed of both smooth and striated muscle. Circular contractile elements are required for sphincteric action and longitudinal smooth muscle may assist by stabilizing the urethra and accentuating force developed by circumferential muscle. The periurethral striated muscle that forms part of the pelvic floor is separate from the intramural striated muscle but together they constitute the EUS. The region of highest urethral pressure occurs approximately 4 cm from the bladder neck in women and in the membranous urethra approximately 5 cm from the neck in men, and pressures of 100–120 cmH₂O are considered normal.

While the bladder is filling, the urethral outlet remains closed and the EUS contracts with greater and greater frequency. This progressive increase in activity of the EUS in the face of bladder expansion is known as the guarding reflex. When the urinary bladder fills to approximately

200–300 ml, stretch receptors in the urinary bladder trigger a reflex arc. The signal stimulates the spinal cord, which responds with a parasympathetic impulse that relaxes the smooth muscle of the internal urethral sphincter and contracts the detrusor muscle. Urine does not flow, however, until a voluntary nerve impulse from the pudendal nerve relaxes the striated muscle of the EUS.

The most common clinical problem encountered with the urethra is blockage. Partial urethral obstruction is relatively common in men with aging, as a result of benign prostate hypertrophy, carcinoma, or calculi, and results in increased back pressure during micturition. During early stages of urethral restriction, which can take years to develop, the bladder undergoes compensatory hypertrophy in order to produce the greater contractile force required to force urine passed the restriction and keep residual urine volumes low (100). These patients present with voiding symptoms, which include urgency, frequency, slowing of the urinary stream, straining to void and nocturia all of which affect quality of life. The primary effects of urethral obstruction are found in the bladder and, if severe enough, will manifest as hydronephrosis and can threaten kidney function (101). Early stage treatment with α -antagonists, such as terazosin, doxazosin, tamsulosin, and alfuzosin, can provide some relief by inhibiting the α_1 -receptor on the smooth muscle within the prostate gland and the internal urethral sphincter; however, this antagonism does not affect the size of the prostate, and the latter requires treatment with a 5 α -reductase inhibitor. α -Antagonists may cause postural hypotension because of the diffuse distribution of receptors along blood vessels. In addition, medical treatment may not always adequately treat symptoms and surgery is then required. If bladder outlet obstruction is left untreated, the bladder wall ultimately decompensates and becomes hyperactive with loss of functional capacity. Alteration in the contractility of the detrusor is accompanied by changes in the proteins that comprise the contractile apparatus and increases in markers of fibrosis such as collagen.

Recent research is beginning to shed light on the complex physiologic interactions between the urothelium, interstitium, smooth muscle, and nervous system. These interactions have highlighted a number of receptors, ion channels, and pathways as possible clinical targets for lower urinary tract symptoms, although the basic understanding of causes remains limited. Indeed, widespread use of the term *lower urinary tract symptoms* (or LUTS) to describe problems such as urgency, frequency, incontinence, and nocturia among others, indicates the shortcomings in our knowledge of causes and is deliberately nonspecific for that reason. Treatment options currently offered include education, behavioral modification, pelvic exercises, medication, botulinum toxin, catheterization, and surgery (often as a result of medication failure or symptom progression). The existence of multiple different guidelines indicates that current therapy of these disorders is empirical, often lacking in an evidence base, and is of inadequate benefit to patients. In clinical practice, the drugs in current use tend to target symptomology with an emphasis on control of smooth muscle motility. In general, these drugs have low efficacy. A major challenge, therefore, is to better understand the molecular and cellular physiology underlying normal functioning of the urinary tract, and greater efforts are needed here. Gaining deeper insights into

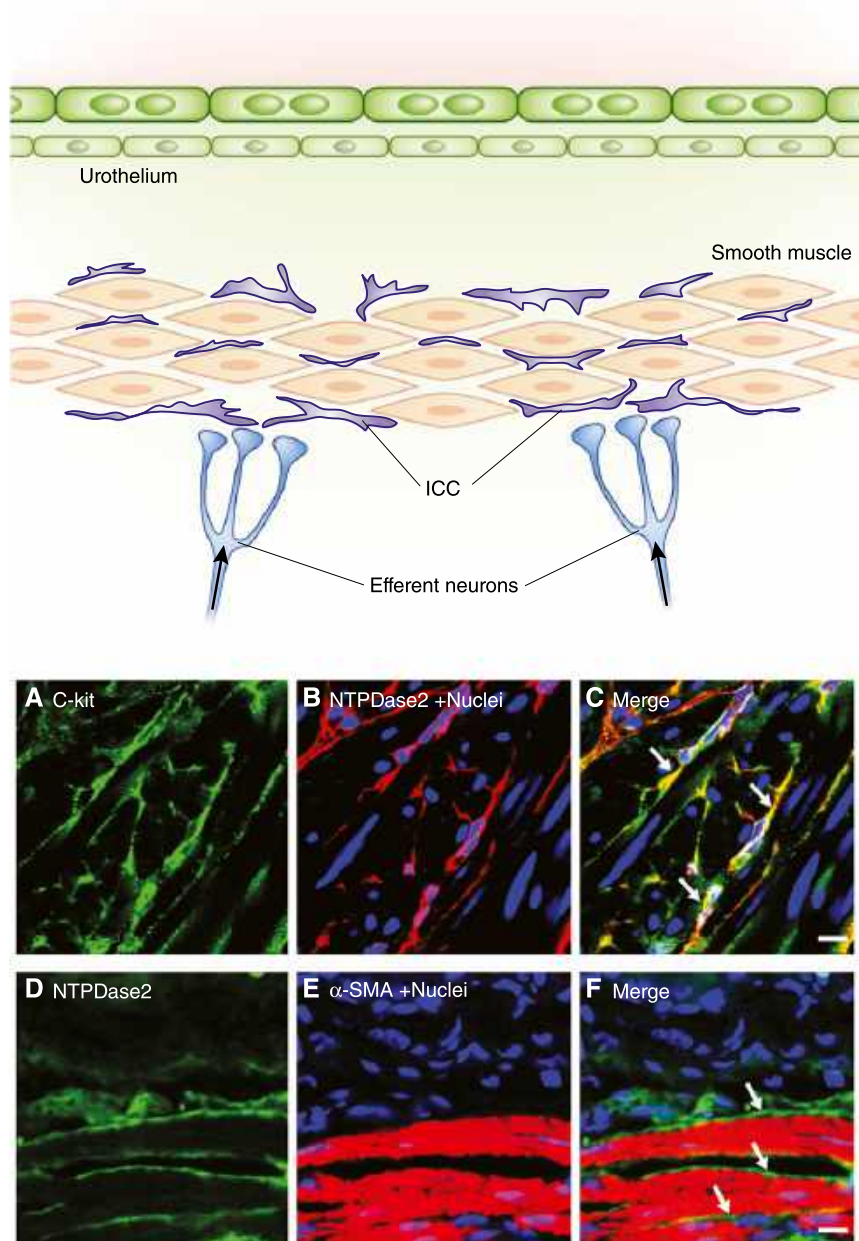


Figure 9. | Morphology and location of BICCs. The top panel provides a schematic of the bladder wall showing the location of ICCs adjacent to smooth muscle bundles and parasympathetic neurons. These stellate-shaped cells are also seen in the deep lamina propria. The lower panels show immunofluorescence staining of BICCs in 5- μ m cryosections of BSM from mouse. (A) c-kit Staining. (B) Ectonucleotidase2 (NTPDase2) staining. (C) Merge showing colocalization (in yellow) of the two BICC markers (arrows). (D–F) images showing close apposition of BICC (arrows) and BSM cells as indicated by NTPDase2 and α -smooth muscle actin. α -SMA, α -smooth muscle actin; BICC, bladder interstitial cell of Cajal; BSM, bladder smooth muscle; ICC, interstitial cell of Cajal. Bar, 10 μ m.

basic physiology will surely lead to improvements in our understanding of the causes of voiding dysfunction and bladder pain syndromes and, thus, will suggest more rational and targeted therapeutic approaches.

Acknowledgments

The author thanks Drs. Mark Zeidel and Melanie Hoenig for their generous assistance with suggestions and constructive criticism during the preparation of this review.

This work was supported by grants from the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (Grants DK08399 and P20-DK097818). Its content is solely the responsibility of the author and does not necessarily represent the official view of the National Institutes of Health.

Disclosures

None.

References

- Tanagho EA, Pugh RC: The anatomy and function of the ureterovesical junction. *Br J Urol* 35: 151–165, 1963
- Raman A, Lam S, Vasilaras A, Joseph D, Wong J, Sved P, Allen RD: Influence of ureteric anastomosis technique on urological complications after kidney transplantation. *Transplant Proc* 45: 1622–1624, 2013
- Dellabella M, Milanese G, Muzzonigro G: Randomized trial of the efficacy of tamsulosin, nifedipine and phloroglucinol in medical expulsive therapy for distal ureteral calculi. *J Urol* 174: 167–172, 2005
- Ye Z, Yang H, Li H, Zhang X, Deng Y, Zeng G, Chen L, Cheng Y, Yang J, Mi Q, Zhang Y, Chen Z, Guo H, He W, Chen Z: A multicentre, prospective, randomized trial: Comparative efficacy of tamsulosin and nifedipine in medical expulsive therapy for distal ureteric stones with renal colic. *BJU Int* 108: 276–279, 2011
- Lang RJ, Hashitani H, Tonta MA, Bourke JL, Parkington HC, Suzuki H: Spontaneous electrical and Ca²⁺ signals in the mouse renal pelvis that drive pyeloureteric peristalsis. *Clin Exp Pharmacol Physiol* 37: 509–515, 2010
- Lang RJ, Hashitani H, Tonta MA, Parkington HC, Suzuki H: Spontaneous electrical and Ca²⁺ signals in typical and atypical smooth muscle cells and interstitial cell of Cajal-like cells of mouse renal pelvis. *J Physiol* 583: 1049–1068, 2007
- Lang RJ, Klemm MF: Interstitial cell of Cajal-like cells in the upper urinary tract. *J Cell Mol Med* 9: 543–556, 2005
- Lang RJ, Tonta MA, Zoltkowski BZ, Meeker WF, Wendt I, Parkington HC: Pyeloureteric peristalsis: Role of atypical smooth muscle cells and interstitial cells of Cajal-like cells as pacemakers. *J Physiol* 576: 695–705, 2006
- Lang RJ, Zoltkowski BZ, Hammer JM, Meeker WF, Wendt I: Electrical characterization of interstitial cells of Cajal-like cells and smooth muscle cells isolated from the mouse ureteropelvic junction. *J Urol* 177: 1573–1580, 2007
- Berridge MJ: Smooth muscle cell calcium activation mechanisms. *J Physiol* 586: 5047–5061, 2008
- Koleda P, Apoznanski W, Wozniak Z, Rusiecki L, Szydelko T, Pilecki W, Polok M, Kalka D, Pupka A: Changes in interstitial cell of Cajal-like cells density in congenital ureteropelvic junction obstruction. *Int Urol Nephrol* 44: 7–12, 2012
- Kuvel M, Canguven O, Murtazaoglu M, Albayrak S: Distribution of Cajal like cells and innervation in intrinsic ureteropelvic junction obstruction. *Arch Ital Urol Androl* 83: 128–132, 2011
- Solari V, Piotrowska AP, Puri P: Altered expression of interstitial cells of Cajal in congenital ureteropelvic junction obstruction. *J Urol* 170: 2420–2422, 2003
- Yang X, Zhang Y, Hu J: The expression of Cajal cells at the obstruction site of congenital pelviureteric junction obstruction and quantitative image analysis. *J Pediatr Surg* 44: 2339–2342, 2009
- Levent A, Büyükaşar K: Expression of Rho-kinase (ROCK-1 and ROCK-2) and its substantial role in the contractile activity of the sheep ureter. *Br J Pharmacol* 143: 431–437, 2004
- Lee HW, Baak CH, Lee MY, Kim YC: Spontaneous contractions augmented by cholinergic and adrenergic systems in the human ureter. *Korean J Physiol Pharmacol* 15: 37–41, 2011
- Malin JM Jr, Deane RF, Boyarsky S: Characterisation of adrenergic receptors in human ureter. *Br J Urol* 42: 171–174, 1970
- Rajpathy J, Aswathaman K, Sinha M, Subramani S, Gopalakrishnan G, Kekre NS: An in vitro study on human ureteric smooth muscle with the alpha1-adrenoceptor subtype blocker, tamsulosin. *BJU Int* 102: 1743–1745, 2008
- Nakada SY, Coyle TL, Ankem MK, Moon TD, Jerde TJ: Doxazosin relaxes ureteral smooth muscle and inhibits epinephrine-induced ureteral contractility in vitro. *Urology* 70: 817–821, 2007
- Al-Ansari A, Al-Naimi A, Alobaidy A, Assadiq K, Azmi MD, Shokeir AA: Efficacy of tamsulosin in the management of lower ureteral stones: A randomized double-blind placebo-controlled study of 100 patients. *Urology* 75: 4–7, 2010
- Park YC, Tomiyama Y, Hayakawa K, Akahane M, Ajisawa Y, Miyatake R, Kiwamoto H, Sugiyama T, Kurita T: Existence of a beta3-adrenoceptor and its functional role in the human ureter. *J Urol* 164: 1364–1370, 2000
- Wanajo I, Tomiyama Y, Yamazaki Y, Kojima M, Shibata N: Pharmacological characterization of beta-adrenoceptor subtypes mediating relaxation in porcine isolated ureteral smooth muscle. *J Urol* 172: 1155–1159, 2004
- Michel MC, Vrydag W: Alpha1-, alpha2- and beta-adrenoceptors in the urinary bladder, urethra and prostate. *Br J Pharmacol* 147[Suppl 2]: S88–S119, 2006
- de Groat WC, Yoshimura N: Afferent nerve regulation of bladder function in health and disease. *Handb Exp Pharmacol* 194: 91–138, 2009
- Yoshimura N, Kaiho Y, Miyazato M, Yunoki T, Tai C, Chancellor MB, Tyagi P: Therapeutic receptor targets for lower urinary tract dysfunction. *Naunyn Schmiedebergs Arch Pharmacol* 377: 437–448, 2008
- Stafford RE, Ashton-Miller JA, Sapsford R, Hodges PW: Activation of the striated urethral sphincter to maintain continence during dynamic tasks in healthy men. *Neurourol Urodyn* 31: 36–43, 2012
- Fowler CJ, Griffiths D, de Groat WC: The neural control of micturition. *Nat Rev Neurosci* 9: 453–466, 2008
- Griffiths D, Derbyshire S, Stenger A, Resnick N: Brain control of normal and overactive bladder. *J Urol* 174: 1862–1867, 2005
- Gandhi D, Molotkov A, Batourina E, Schneider K, Dan H, Reiley M, Laufer E, Metzger D, Liang F, Liao Y, Sun TT, Aronow B, Rosen R, Mauney J, Adam R, Rosselot C, Van Batavia J, McMahon A, McMahon J, Guo JJ, Mendelsohn C: Retinoid signaling in progenitors controls specification and regeneration of the urothelium. *Dev Cell* 26: 469–482, 2013
- Wu XR, Kong XP, Pellicer A, Kreibich G, Sun TT: Uroplakins in urothelial biology, function, and disease. *Kidney Int* 75: 1153–1165, 2009
- Hu P, Deng FM, Liang FX, Hu CM, Auerbach AB, Shapiro E, Wu XR, Kachar B, Sun TT: Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. *J Cell Biol* 151: 961–972, 2000
- Hu P, Meyers S, Liang FX, Deng FM, Kachar B, Zeidel ML, Sun TT: Role of membrane proteins in permeability barrier function: Uroplakin ablation elevates urothelial permeability. *Am J Physiol Renal Physiol* 283: F1200–F1207, 2002
- Min G, Zhou G, Schapira M, Sun TT, Kong XP: Structural basis of urothelial permeability barrier function as revealed by Cryo-EM studies of the 16 nm uroplakin particle. *J Cell Sci* 116: 4087–4094, 2003
- Lavelle JP, Meyers SA, Ruiz WG, Buffington CA, Zeidel ML, Apodaca G: Urothelial pathophysiological changes in feline interstitial cystitis: A human model. *Am J Physiol Renal Physiol* 278: F540–F553, 2000
- Apodaca G: The uroepithelium: Not just a passive barrier. *Traffic* 5: 117–128, 2004
- Apodaca G, Balestreire E, Birder LA: The uroepithelial-associated sensory web. *Kidney Int* 72: 1057–1064, 2007
- Gosling JA: Modification of bladder structure in response to outflow obstruction and ageing. *Eur Urol* 32[Suppl 1]: 9–14, 1997
- Macarak EJ, Howard PS: The role of collagen in bladder filling. *Adv Exp Med Biol* 462: 215–223, discussion 225–233, 1999
- Ferguson DR, Kennedy I, Burton TJ: ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes—a possible sensory mechanism? *J Physiol* 505: 503–511, 1997
- Lewis SA, Lewis JR: Kinetics of urothelial ATP release. *Am J Physiol Renal Physiol* 291: F332–F340, 2006
- Schnegelsberg B, Sun TT, Cain G, Bhattacharya A, Nunn PA, Ford AP, Vizzard MA, Cockayne DA: Overexpression of NGF in mouse urothelium leads to neuronal hyperinnervation, pelvic sensitivity, and changes in urinary bladder function. *Am J Physiol Regul Integr Comp Physiol* 298: R534–R547, 2010
- Kanasaki K, Yu W, von Bodungen M, Larigakis JD, Kanasaki M, Ayala de la Pena F, Kalluri R, Hill WG: Loss of β 1-integrin from urothelium results in overactive bladder and incontinence in mice: A mechanosensory rather than structural phenotype. *FASEB J* 27: 1950–1961, 2013
- Iacovou JW, Hill SJ, Birmingham AT: Agonist-induced contraction and accumulation of inositol phosphates in the guinea-pig detrusor: Evidence that muscarinic and purinergic receptors raise intracellular calcium by different mechanisms. *J Urol* 144: 775–779, 1990

44. Ding HL, Ryder JW, Stull JT, Kamm KE: Signaling processes for initiating smooth muscle contraction upon neural stimulation. *J Biol Chem* 284: 15541–15548, 2009
45. Hegde SS: Muscarinic receptors in the bladder: From basic research to therapeutics. *Br J Pharmacol* 147[Suppl 2]: S80–S87, 2006
46. Uchiyama T, Chess-Williams R: Muscarinic receptor subtypes of the bladder and gastrointestinal tract. *J Smooth Muscle Res* 40: 237–247, 2004
47. Zhang X, DiSanto ME: Rho-kinase, a common final path of various contractile bladder and ureter stimuli. *Handb Exp Pharmacol* 202: 543–568, 2011
48. Lee HY, Bardini M, Burnstock G: Distribution of P2X receptors in the urinary bladder and the ureter of the rat. *J Urol* 163: 2002–2007, 2000
49. Vial C, Evans RJ: P2X receptor expression in mouse urinary bladder and the requirement of P2X(1) receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle. *Br J Pharmacol* 131: 1489–1495, 2000
50. Yu W, Sun X, Robson SC, Hill WG: Extracellular UDP enhances P2X-mediated bladder smooth muscle contractility via P2Y6 activation of the phospholipase C/inositol trisphosphate pathway. *FASEB J* 27: 1895–1903, 2013
51. Yu W, Robson SC, Hill WG: Expression and distribution of ectonucleotidases in mouse urinary bladder. *PLoS ONE* 6: e18704, 2011
52. Maserejian NN, Wager CG, Giovannucci EL, Curto TM, McVary KT, McKinlay JB: Intake of caffeinated, carbonated, or citrus beverage types and development of lower urinary tract symptoms in men and women. *Am J Epidemiol* 177: 1399–1410, 2013
53. Lohsiriwat S, Hirunsai M, Chaiyaprasithi B: Effect of caffeine on bladder function in patients with overactive bladder symptoms. *Urol Ann* 3: 14–18, 2011
54. Kershen R, Mann-Gow T, Yared J, Stromberg I, Zvara P: Caffeine ingestion causes detrusor overactivity and afferent nerve excitation in mice. *J Urol* 188: 1986–1992, 2012
55. Yoshida M, Miyamae K, Iwashita H, Otani M, Inadome A: Management of detrusor dysfunction in the elderly: Changes in acetylcholine and adenosine triphosphate release during aging. *Urology* 63[Suppl 1]: 17–23, 2004
56. Yoshida M, Homma Y, Inadome A, Yono M, Seshita H, Miyamoto Y, Murakami S, Kawabe K, Ueda S: Age-related changes in cholinergic and purinergic neurotransmission in human isolated bladder smooth muscles. *Exp Gerontol* 36: 99–109, 2001
57. Sjögren C, Andersson KE, Husted S, Mattiasson A, Møller-Madsen B: Atropine resistance of transmurally stimulated isolated human bladder muscle. *J Urol* 128: 1368–1371, 1982
58. Andersson KE, Arner A: Urinary bladder contraction and relaxation: Physiology and pathophysiology. *Physiol Rev* 84: 935–986, 2004
59. Michel MC, Barendrecht MM: Physiological and pathological regulation of the autonomic control of urinary bladder contractility. *Pharmacol Ther* 117: 297–312, 2008
60. Frazier EP, Mathy MJ, Peters SL, Michel MC: Does cyclic AMP mediate rat urinary bladder relaxation by isoproterenol? *J Pharmacol Exp Ther* 313: 260–267, 2005
61. Frazier EP, Peters SL, Braverman AS, Ruggieri MR Sr, Michel MC: Signal transduction underlying the control of urinary bladder smooth muscle tone by muscarinic receptors and beta-adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 377: 449–462, 2008
62. Hristov KL, Chen M, Kellett WF, Rovner ES, Petkov GV: Large-conductance voltage- and Ca²⁺-activated K⁺ channels regulate human detrusor smooth muscle function. *Am J Physiol Cell Physiol* 301: C903–C912, 2011
63. Hristov KL, Cui X, Brown SM, Liu L, Kellett WF, Petkov GV: Stimulation of beta3-adrenoceptors relaxes rat urinary bladder smooth muscle via activation of the large-conductance Ca²⁺-activated K⁺ channels. *Am J Physiol Cell Physiol* 295: C1344–C1353, 2008
64. Petkov GV, Nelson MT: Differential regulation of Ca²⁺-activated K⁺ channels by beta-adrenoceptors in guinea pig urinary bladder smooth muscle. *Am J Physiol Cell Physiol* 288: C1255–C1263, 2005
65. Afeli SA, Rovner ES, Petkov GV: SK but not IK channels regulate human detrusor smooth muscle spontaneous and nerve-evoked contractions. *Am J Physiol Renal Physiol* 303: F559–F568, 2012
66. Chen M, Kellett WF, Petkov GV: Voltage-gated K(+) channels sensitive to stromatolxin-1 regulate myogenic and neurogenic contractions of rat urinary bladder smooth muscle. *Am J Physiol Regul Integr Comp Physiol* 299: R177–R184, 2010
67. Hristov KL, Chen M, Afeli SA, Cheng Q, Rovner ES, Petkov GV: Expression and function of K(V)2-containing channels in human urinary bladder smooth muscle. *Am J Physiol Cell Physiol* 302: C1599–C1608, 2012
68. Petkov GV, Heppner TJ, Bonev AD, Herrera GM, Nelson MT: Low levels of K(ATP) channel activation decrease excitability and contractility of urinary bladder. *Am J Physiol Regul Integr Comp Physiol* 280: R1427–R1433, 2001
69. Persson K, Alm P, Johansson K, Larsson B, Andersson KE: Nitric oxide synthase in pig lower urinary tract: Immunohistochemistry, NADPH diaphorase histochemistry and functional effects. *Br J Pharmacol* 110: 521–530, 1993
70. Dixon JS, Jen PY: Development of nerves containing nitric oxide synthase in the human male urogenital organs. *Br J Urol* 76: 719–725, 1995
71. Hedlund P: Nitric oxide/cGMP-mediated effects in the outflow region of the lower urinary tract—is there a basis for pharmacological targeting of cGMP? *World J Urol* 23: 362–367, 2005
72. Uckert S, Oelke M: Phosphodiesterase (PDE) inhibitors in the treatment of lower urinary tract dysfunction. *Br J Clin Pharmacol* 72: 197–204, 2011
73. Müntener M, Schurch B, Wefer B, Reitz A: Systemic nitric oxide augmentation leads to a rapid decrease of the bladder outlet resistance in healthy men. *Eur Urol* 50: 112–117, discussion 117–118, 2006
74. Canda AE: Diabetes might adversely affect expression and function of interstitial cells in the urinary bladder and urethra in humans: A new mechanism in the development of diabetic lower urinary dysfunction? *Med Hypotheses* 76: 632–634, 2011
75. Drake M: Interstitial cells of cajal in the human normal urinary bladder and in the bladder of patients with megacystis-microcolon intestinal hypoperistalsis syndrome. *BJU Int* 94: 1402, 2004
76. Hashitani H, Yanai Y, Suzuki H: Role of interstitial cells and gap junctions in the transmission of spontaneous Ca²⁺ signals in detrusor smooth muscles of the guinea-pig urinary bladder. *J Physiol* 559: 567–581, 2004
77. Johnston L, Carson C, Lyons AD, Davidson RA, McCloskey KD: Cholinergic-induced Ca²⁺ signaling in interstitial cells of Cajal from the guinea pig bladder. *Am J Physiol Renal Physiol* 294: F645–F655, 2008
78. Johnston L, Cunningham RM, Young JS, Fry CH, McMurray G, Eccles R, McCloskey KD: Altered distribution of interstitial cells and innervation in the rat urinary bladder following spinal cord injury. *J Cell Mol Med* 16: 1533–1543, 2012
79. Kim SO, Song SH, Ahn KY, Kwon DD: Distribution of interstitial cells of cajal in menopausal rat urinary bladder showing detrusor overactivity. *Int Neurourol J* 14: 48–53, 2010
80. Kubota Y, Kojima Y, Shibata Y, Imura M, Sasaki S, Kohri K: Role of KIT-positive interstitial cells of cajal in the urinary bladder and possible therapeutic target for overactive bladder. *Adv Urol* 2011: 816342, 2011
81. McCloskey KD: Interstitial cells in the urinary bladder—localization and function. *Neurourol Urodyn* 29: 82–87, 2010
82. Won KJ, Sanders KM, Ward SM: Interstitial cells of Cajal mediate mechanosensitive responses in the stomach. *Proc Natl Acad Sci U S A* 102: 14913–14918, 2005
83. Choi KM, Gibbons SJ, Roeder JL, Lurken MS, Zhu J, Wouters MM, Miller SM, Szurszewski JH, Farrugia G: Regulation of interstitial cells of Cajal in the mouse gastric body by neuronal nitric oxide. *Neurogastroenterol Motil* 19: 585–595, 2007
84. Ward SM, Beckett EA, Wang X, Baker F, Khoyi M, Sanders KM: Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. *J Neurosci* 20: 1393–1403, 2000
85. Nakayama S, Kajioka S, Goto K, Takaki M, Liu HN: Calcium-associated mechanisms in gut pacemaker activity. *J Cell Mol Med* 11: 958–968, 2007
86. Streutker CJ, Huizinga JD, Driman DK, Riddell RH: Interstitial cells of Cajal in health and disease. Part I: normal ICC structure

- and function with associated motility disorders. *Histopathology* 50: 176–189, 2007
87. Mostafa RM, Moustafa YM, Hamdy H: Interstitial cells of Cajal, the Maestro in health and disease. *World J Gastroenterol* 16: 3239–3248, 2010
 88. Yu W, Zeidel ML, Hill WG: Cellular expression profile for interstitial cells of cajal in bladder - a cell often misidentified as myocyte or myofibroblast. *PLoS ONE* 7: e48897, 2012
 89. Lagou M, De Vente J, Kirkwood TB, Hedlund P, Andersson KE, Gillespie JI, Drake MJ: Location of interstitial cells and neurotransmitters in the mouse bladder. *BJU Int* 97: 1332–1337, 2006
 90. Johnston L, Woolsey S, Cunningham RM, O’Kane H, Duggan B, Keane P, McCloskey KD: Morphological expression of KIT positive interstitial cells of Cajal in human bladder. *J Urol* 184: 370–377, 2010
 91. Davidson RA, McCloskey KD: Morphology and localization of interstitial cells in the guinea pig bladder: Structural relationships with smooth muscle and neurons. *J Urol* 173: 1385–1390, 2005
 92. Piaseczna Piotrowska A, Rolle U, Solari V, Puri P: Interstitial cells of Cajal in the human normal urinary bladder and in the bladder of patients with megacystis-microcolon intestinal hypoperistalsis syndrome. *BJU Int* 94: 143–146, 2004
 93. Rolle U, Puri P: Structural basis of voiding dysfunction in megacystis microcolon intestinal hypoperistalsis syndrome. *J Pediatr Urol* 2: 277–284, 2006
 94. Okada S, Kojima Y, Kubota Y, Mizuno K, Sasaki S, Kohri K: Attenuation of bladder overactivity in KIT mutant rats. *BJU Int* 108: E97–E103, 2011
 95. McCloskey KD, Anderson UA, Davidson RA, Bayguinov YR, Sanders KM, Ward SM: Comparison of mechanical and electrical activity and interstitial cells of Cajal in urinary bladders from wild-type and W/W^v mice. *Br J Pharmacol* 156: 273–283, 2009
 96. Biers SM, Reynard JM, Doore T, Brading AF: The functional effects of a c-kit tyrosine inhibitor on guinea-pig and human detrusor. *BJU Int* 97: 612–616, 2006
 97. Hashitani H: Interaction between interstitial cells and smooth muscles in the lower urinary tract and penis. *J Physiol* 576: 707–714, 2006
 98. Sui GP, Rothery S, Dupont E, Fry CH, Severs NJ: Gap junctions and connexin expression in human suburothelial interstitial cells. *BJU Int* 90: 118–129, 2002
 99. Fry CH, Sui GP, Severs NJ, Wu C: Spontaneous activity and electrical coupling in human detrusor smooth muscle: Implications for detrusor overactivity? *Urology* 63[Suppl 1]: 3–10, 2004
 100. Zderic SA, Chacko S: Alterations in the contractile phenotype of the bladder: Lessons for understanding physiological and pathological remodelling of smooth muscle. *J Cell Mol Med* 16: 203–217, 2012
 101. Zeidel ML: Obstructive uropathy. In: *Goldman’s Cecil Medicine*, edited by Goldman L, Schafer AI, 24th Ed., Philadelphia, Elsevier, 2011, pp 776–780
 102. Yoshimura N, Chancellor MB: Physiology and pharmacology of the bladder and urethra. In: *Campbell-Walsh Urology*, 10th Ed., Philadelphia, Elsevier Saunders, 2012, pp 1786–1833.e17

Published online ahead of print. Publication date available at www.cjasn.org.



Integrated Control of Na Transport along the Nephron

Lawrence G. Palmer* and Jürgen Schnemann†

Abstract

The kidney filters vast quantities of Na at the glomerulus but excretes a very small fraction of this Na in the final urine. Although almost every nephron segment participates in the reabsorption of Na in the normal kidney, the proximal segments (from the glomerulus to the macula densa) and the distal segments (past the macula densa) play different roles. The proximal tubule and the thick ascending limb of the loop of Henle interact with the filtration apparatus to deliver Na to the distal nephron at a rather constant rate. This involves regulation of both filtration and reabsorption through the processes of glomerulotubular balance and tubuloglomerular feedback. The more distal segments, including the distal convoluted tubule (DCT), connecting tubule, and collecting duct, regulate Na reabsorption to match the excretion with dietary intake. The relative amounts of Na reabsorbed in the DCT, which mainly reabsorbs NaCl, and by more downstream segments that exchange Na for K are variable, allowing the simultaneous regulation of both Na and K excretion.

Clin J Am Soc Nephrol 10: 676–687, 2015. doi: 10.2215/CJN.12391213

Introduction

Daily Na intake in the United States averages approximately 180 mmol (4.2 g) for men and 150 mmol (3.5 g) for women (1). Because Na is an immutable ion, mass balance requires that an equal amount must be removed from the body daily to prevent inappropriate gains or losses of Na and its accompanying anions, chloride and bicarbonate. Because these ions are the prime determinants of extracellular fluid volume, maintenance of extracellular fluid volume, arterial blood pressure, and organ perfusion depends on control of body Na content.

Under most conditions, the kidneys excrete >95% of the ingested Na at rates that match dietary Na intake. Under steady-state conditions, this process is remarkably precise in both healthy and diseased kidneys, and determinations of excretion are used to estimate Na intake (e.g., in determining adherence to a prescribed dietary regimen). Recent studies have shown the presence of a sizable subcutaneous Na pool that is not in solution equilibrium with the freely exchangeable extracellular Na. Long-term observations suggest that cyclical release of Na from this pool can lead to excretion rates that deviate from Na intake (2). The speed of achieving an intake/excretion match or a new steady state when Na intake varies is relatively slow; body Na content usually increases somewhat when Na intake increases and decreases somewhat when Na intake decreases (3). These changes in body Na content and blood volume are the likely cause for the relation between Na intake and BP. In normotensive individuals, the effect of variations in daily Na intake on BP is small, about 2 mmHg for a 50% reduction in Na intake (4). Nevertheless, such a reduction is associated with demonstrable health benefits, particularly in salt-sensitive persons, such as those with hypertension, patients with diabetes, African Americans, and individuals with chronic renal disease (4).

The precise adaptation of urinary Na excretion to dietary Na intake results from regulated processing of an ultrafiltrate of circulating plasma by the renal tubular epithelium. Perhaps the most striking aspect of this remarkable process is the gross inequality in the quantities of Na removed from plasma by ultrafiltration and those removed from the body by urinary excretion. The staggering mass of >500 g of Na is extracted daily from the plasma by ultrafiltration in order to excrete the ingested 3 g. The large filtered load results from the high extracellular Na concentration and the high rate of glomerular ultrafiltration. Whatever the evolutionary pressure behind this functional design may be, the necessity to retrieve almost all of the filtered Na before it reaches the urine represents a challenging regulatory and energetic demand that the tubular epithelium has to meet. Just as Na intake dictates the rate of Na excretion, Na filtration dictates the rate of Na reabsorption.

Na Reabsorption along the Nephron

Micropuncture and microperfusion studies have shown that all nephron segments contribute to the retrieval of filtered Na (with the exception of the thin descending limbs of the loop of Henle) (Figure 1). The reabsorption of Na is an energy-consuming process that is powered by a Na⁺- and K⁺-activated ATPase in the basolateral membranes of all Na-reabsorbing cells in the kidney. Oxygen consumption of the kidneys is similar to that of other major organs (approximately 6–8 ml/min per 100 g) and is extracted from a seemingly excessive blood supply. While the kidneys consume between 7% and 10% of total oxygen uptake, they receive about 20%–25% of cardiac output at rest. Two thirds or more of renal oxygen uptake are expended for the needs of the Na⁺K-ATPase, which is for active Na reabsorption.

*Department of Physiology and Biophysics, Weill-Cornell Medical College, New York, New York; and †Kidney Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

Correspondence:

Dr. Lawrence G. Palmer, Department of Physiology and Biophysics, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10065. Email: lgpalm@med.cornell.edu

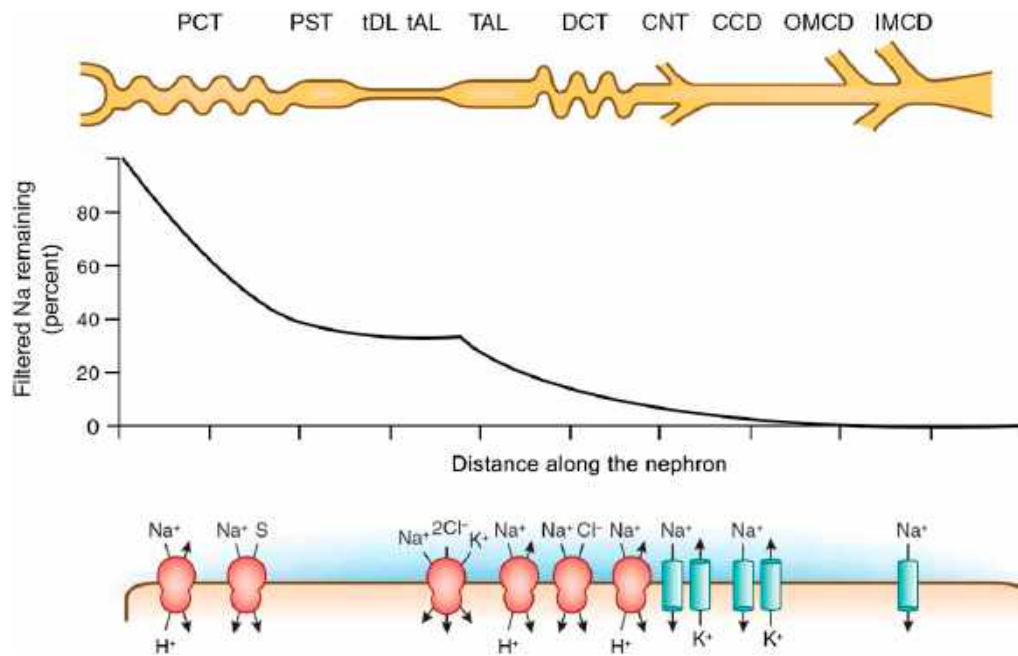


Figure 1. | Na transport along the nephron. The fraction of Na remaining in the ultrafiltrate is plotted as a function of distance along the nephron under conditions of normal (approximately 100 mmol/d) salt intake. The cartoon indicates the major nephron segments. The symbols below show the key Na transport mechanisms in each segment. CCD, cortical collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; IMCD, inner medullary collecting duct; OMCD, outer medullary collecting duct; PCT, proximal convoluted tubule; PST, proximal straight tubule; S, solute (various); tAL, thin ascending limb; TAL, thick ascending limb; tDL, thin descending limb.

As much as 60%–70% of total Na reabsorption takes place along the proximal convoluted tubule (PCT) and proximal straight tubule, and because reabsorption is near isotonic in this part of the nephron, this is also true for the reabsorption of water. While the thin descending limbs of the loop of Henle absorb water, but not Na, another 25%–30% of the filtered Na is reabsorbed by the ascending portion of this loop, mostly the thick limbs (TALH). Thus, by the time the filtered fluid reaches the macula densa cells at the transition to the distal convoluted tubule (DCT), about 90% of the filtered Na has been retrieved. In quantitative terms, Na reabsorption, therefore, is mostly a function of the proximal nephron (proximal tubule and loop of Henle) while the DCT and the cortical (CCD) and medullary (MCD) collecting ducts contribute no more than 5%–10% of total Na reabsorption. Nevertheless, because of the magnitude of the rate of ultrafiltration, 10% of the total daily filtered load is still an amount that is in the order of the rapidly exchangeable extracellular Na (about 60 g). In fact, it is Na reabsorption along the distal nephron that is highly regulated, and failure of the distal nephron to reabsorb Na is generally more deleterious to Na homeostasis than proximal nephron malabsorption.

Rates and Mechanisms of Na Transport along the Nephron

Proximal Tubule

The renal proximal tubule is a prototypical low-resistance epithelium characterized by low transepithelial voltage, high ion permeabilities, constitutively high water permeability,

low transepithelial osmotic gradients, and near-isotonic fluid transport. Results from micropuncture studies indicate avid reabsorption of Na without measurable changes in Na concentration along the PCT. The magnitude of Na reabsorption per millimeter of nephron length in the rat is on the order of 300 pEq/min in PCT from superficial nephrons and even higher in PCTs from juxtamedullary nephrons. Transport rates along the proximal tubule decrease substantially with distance from the glomerulus, and this is accompanied by reductions in the number of mitochondria and the extent of surface membrane amplification both apically and basolaterally. For example, micropuncture studies have shown that fluid and, presumably, Na reabsorption in the rat fell by 75% over the initial 5 mm of proximal tubule length (5). As discussed in a previous article in this series, all Na absorption in the PCT is driven directly or indirectly by the action of the basolateral Na,K-ATPase (6). Active translocation of Na creates driving forces for Na-dependent cotransport and ion exchange, as well as diffusive gradients for paracellular ion movement. Quantitatively, apical Na/H exchange mediated by NHE3 is the most important reabsorptive mechanism. It mediates NaHCO_3 uptake, generates an electrochemical gradient for paracellular salt absorption, and operates in tandem with Cl/base exchange in the later part of the proximal tubule. Active transport, electrodiffusion, and solvent drag each contribute approximately one third to total Na reabsorption in the proximal tubule (7).

The discovery of claudin2 as a tight junction protein highly expressed along the PCT has greatly improved the understanding of the paracellular shunt properties in this

part of the nephron. Expression studies in Madin-Darby canine kidney cells and the use of claudin2-deficient mice strongly indicate that claudin2 provides a cation-selective and water-permeable paracellular conductance in the PCT (8–11). Claudin2 appears to be a major molecular contributor to the low-resistance properties of the proximal epithelium.

The contributions of Na-dependent cotransporters linked to glucose, phosphate, amino acids, lactate, and other molecules to apical Na uptake are small. For example, with a glucose-to-Na stoichiometry of 1:1 and complete glucose reabsorption along the PCT, <5% of Na uptake along the PCT would be mediated by the Na/glucose cotransporter SGLT2.

Loop of Henle

The loop of Henle is heterogeneous, consisting of the thin descending limbs, the thin ascending limbs, and TALH. Thin descending limbs are inhomogeneous in both structural and functional aspects and also vary substantially between species. Generalizable characteristics include a very high water permeability due to the presence of AQP1, low permeabilities for Na and urea in the outer medulla with increasing values in the inner medulla, and very low levels of Na,K-ATPase activity throughout (12). Thus, this segment is unlikely to contribute measurably to renal Na retrieval. Its function is to be seen in the context of the renal concentrating mechanism. Thin ascending limbs reabsorb some Na, but because Na,K-ATPase levels are also very low, this absorption is presumably passive. Compared with the thin descending limbs of the loop of Henle, thin ascending limbs are more permeable to Na and urea and have 100-fold lower water permeability (12).

In contrast, TALH is a major Na-reabsorbing segment accounting for about 25%–30% of renal net Na retrieval. The Na/K/2Cl cotransporter NKCC2 and NHE3 are the predominant mechanisms of Na transport in the TALH (13). Na reabsorption in the absence of measurable water permeability is an essential prerequisite for the ability of the kidney to osmotically concentrate the urine above isotonicity (12).

Macula densa (MD) cells, a subset of 15–25 cells per nephron at the distal end of the TALH, share most transport properties with proper TALH cells even though they are distinct in other aspects. Na uptake is mostly through the A and B isoforms of NKCC2 with some contribution of NHE2, while Na extrusion is mediated by Na,K-ATPase. K recycling across apical renal outer medullary K channel and basolateral exit of Cl through a Cl channel also mimic TALH properties. In MD cells, enhanced uptake through NKCC2 is coupled to the generation of a vasoconstrictor signal to the afferent arterioles that mediates the tubuloglomerular feedback response (14). This signal entails the release of ATP into the juxtaglomerular interstitium and the subsequent generation of the nucleoside adenosine that constricts afferent arterioles through activation of A1 adenosine receptors. Furosemide and other NKCC2 inhibitors interrupt the MD signaling pathway and, therefore, uncouple the elevated NaCl concentrations from their constrictor effect on the preglomerular vasculature (15). The absence of a tubuloglomerular feedback-mediated suppression of GFR

enhances the natriuretic effect of furosemide and the salt-losing phenotype of disease-causing mutations of NKCC2.

Distal Convoluted Tubule

Micropuncture measurements indicate that about 8%–10% of filtered Na enters the initial 20% of the distal convoluted tubule (16–18). At the most distal site that is accessible to micropuncture—just before the first coalescence of tubules to form the collecting duct—about 0.5%–2% of the filtered load remains in the urine. Thus, this part of the nephron reabsorbs 6%–10% of the filtered Na. However, the DCT as defined by these micropuncture-accessible points is heterogeneous, consisting of several different segments that can be distinguished anatomically as DCT1, DCT2, and the connecting tubule (CNT) (19).

The mechanisms of Na transport are different in the “early” or more proximal DCT (mainly DCT1) and in the “late DCT” (DCT2+CNT). In the early DCT, Na reabsorption is largely sensitive to thiazide diuretics, implicating the NaCl-cotransporter NCC. In addition, this segment can reabsorb HCO_3^- through an Na-H exchange process mediated by NHE2 (20), although this accounts for only about 10% of Na reabsorption in the DCT (21,22). In the late DCT, Na reabsorption is sensitive to amiloride (16), indicating transport through the epithelial Na channel (ENaC). A limited number of measurements of isolated perfused CNTs of rabbits (23,24), as well as patch-clamp measurements in rats and mice (25,26), are in accord with this picture. Immunocytochemistry has confirmed the physiologic and pharmacologic data in that NCC expression diminishes in the distal direction (from DCT1 to DCT2 to CNT) while that of ENaC increases (27).

Collecting Duct

The collecting duct, which is divided into the CCD, outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD), handles only about 1% of filtered Na according to micropuncture analysis. However, transport of Na here is remarkable for two reasons. First, it is highly variable, helping to match Na excretion with Na intake. Second, the collecting duct can reduce urine Na concentrations to very low levels (<1 mM), establishing large transtubular Na gradients.

CCDs from rats fed normal chow transport very little Na *in vitro* (28,29). However, if the animals are pretreated with an Na-deficient diet or with mineralocorticoids, net fluxes of 35–100 pEq/mm per minute can be measured. Direct measurements of ENaC activity in principal cells suggest that Na channels form the apical entry step in these segments (25,30,31). Recent work has indicated that the Na-dependent Cl-HCO₃ exchanger (SLC4A8), operating in parallel with the Cl-HCO₃ exchanger pendrin, may provide an alternative pathway (32). Na channel densities are similar in the rat CCD and OMCD (33), indicating that transport capacities are similar in these two segments. In the rabbit, however, Na transport rates in the OMCD were lower (34).

Na transport in the IMCD is controversial. Although one study of isolated IMCD segments detected no net Na transport (35), others have found robust Na reabsorption (up to 140 pEq/mm per minute) (36,37). In the latter case, the transport mechanism differed from that of the rest of the

collecting duct in that it was not associated with a negative lumen voltage. It was inhibited by the loop diuretic furosemide, consistent with a role of the triple cotransporter (NKCC2) as in the TALH. Finally, studies of the IMCD *in situ* using split-drop techniques (38) or microcatheterization (39) indicated even larger transport rates of up to 240 pEq/mm per minute, similar to those in the DCT. This reabsorption was partially inhibited by amiloride and sensitive to the mineralocorticoid state of the animal, similar to that in the CCD. However electrophysiologic measurements suggested a low ENaC activity in these segments (33). Thus, the rate and mechanism of Na reabsorption in the IMCD, as well as its role in Na homeostasis, are uncertain.

Urine Na Concentration

Rats fed a diet very low in Na can reduce the concentration of Na⁺ in urine to <1 mM. Humans are also capable of this degree of Na⁺ scavenging, as illustrated by the Yanomamo people of the Amazon basin whose diet is nearly devoid of Na and whose urinary Na excretion rates are <1 mmol/d (40). How and where this very dilute salt solution is produced is not entirely clear. The rabbit CCD is able to reduce luminal Na⁺ concentration to approximately 10–15 mM *in vitro* from an initial perfusate with 140 mM under conditions of very low flow rates (41). Further reductions of these levels could occur in the OMCD and IMCD if the paracellular resistance is sufficiently large to maintain Na gradients of this magnitude. In fact, measurements of IMCD segments *in vitro* indicate rather high rates of Na leak (approximately 100 pEq/min per millimeter) and low transepithelial resistance (about 50 Ω per cm²) (35,37,39,42,43). In contrast, epithelia known to produce or sustain very large Na concentration gradients, such as urinary bladder, have much higher junctional resistances (44–46). It is possible that the observed leak conductances in the IMCD result from tissue damage during the preparation or perfusion procedure. Alternatively, transport rates would need to be very high to maintain low Na concentrations in the relatively leaky tubules.

Flow Dependence of Na Transport

A basic form of communication between parts of the nephron is through changes in Na delivery that result from changes in GFR for the proximal tubule, and from changes in reabsorption in an upstream segment in all other parts of the nephron. In general, a change in delivery provokes a change in downstream absorption in the same direction, thereby blunting the effect of changes in Na input on the output to the downstream segment.

Proximal Tubule–Glomerulotubular Balance

Na reabsorption in the proximal tubule is highly and directly dependent on the rate of filtration, a phenomenon referred to as glomerulotubular balance (GTB). Adapting reabsorption to filtration has the dual function of minimizing Na and fluid losses when GFR increases, and of preventing cessation of tubular flow with decreasing GFR. The term GTB originally defined the relation between GFR and total urinary Na excretion (47). Numerous studies have shown that changes in GFR are accompanied by nearly proportional changes in tubular reabsorption so that Na excretion and

fractional Na reabsorption, the reabsorption rate expressed as percentage of filtered rate, change very little with decreases or increases of GFR (48,49).

The existence of GTB in the PCT has been shown in numerous micropuncture and microperfusion studies in which variations of GFR were spontaneous or were induced by experimental interventions, such as reductions of renal perfusion pressure. The degree of GTB was often near-perfect, although some deviations from proportionality have been observed during low GFRs (48,50,51). Although GTB in the PCT is a robust and easily demonstrable phenomenon, its explanation has remained a challenge. Studies in intact animals support the notion that flow dependence of proximal fluid reabsorption is the result of changes in physical forces, such as hydrostatic and oncotic pressures in the peritubular capillary bed. Changes in proximal fluid reabsorption that correlate positively with changes in peritubular net absorption pressure have been observed during reduction of renal perfusion pressure, extracellular volume expansion, renal venous pressure elevation, and arterial hypertension (52–55).

An alternative explanation posits that reabsorbed substrates linked to Na reabsorption, such as glucose, amino acids, organic acids, and bicarbonate, are depleted faster along the proximal tubules at low, compared with high, flow rates, and that this would create flow dependence of Na uptake (56,57). Finally, studies in perfused tubules under a variety of conditions seem to indicate that tubular flow rate *per se*, independent of peritubular force variations or compositional changes, can directly affect Na and fluid reabsorption (58). In recent studies, flow-dependent reabsorption could be described by a model in which the bending moment or torque at the level of the microvilli acts as the signal for reabsorption (59). This is supported by the observation that increasing perfusion fluid viscosity, and, therefore, fluid shear stress, was associated with increased reabsorption over the entire flow range studied. In cultured proximal tubule cells, exposure to shear stress induces trafficking of NHE3 to the apical membrane, and this process can be prevented by disruption of cytoskeletal organization (60). Whether the central cilium, an epithelial cell organelle with the demonstrated ability to function as a flow sensor in a number of cells, could be involved in the regulation of proximal tubular reabsorption is an interesting but untested possibility.

Tubuloglomerular Feedback

Reabsorption of the filtered Na load along the proximal tubule in proportion to GFR implies that rates of Na and fluid escaping absorption will also change in proportion to their rates of filtration. Thus, despite GTB, delivery to the tubular segments beyond the proximal tubule will increase whenever GFR increases. Furthermore, GTB would be unable to compensate for a primary impairment of proximal Na reabsorption due to transporter malfunction or dysregulation. Thus, Na homeostasis would be well served by a mechanism that senses an increase in NaCl delivery to the late nephron and would use this information to initiate a counter-regulatory response. Extensive research has established that the MD cells situated near the transition from the TALH to the DCT act as sensors of NaCl concentration, and that their output affects the tone of the afferent glomerular arterioles

and, therefore, GFR (14). The assignment of the MD cells to this specific role is based on the cyto-architectural feature of a consistent and close anatomic contact between MD cells and glomerular arterioles in a region called the juxtaglomerular apparatus (Figure 2). As discussed below, NaCl concentration at the site of the MD cells is an indicator of GFR or of flow rate at the end of the proximal tubule because tubular flow rate is converted into a concentration signal by the function of the TALH (61). GTB and tubuloglomerular feedback are interdependent and arranged in a feedback circuit such that the fluid escaping absorption along the proximal tubule can exert negative control over GFR formation (62). In this circuit it is irrelevant whether the change in NaCl concentration at the sensor site is the result of a change in GFR or in proximal reabsorption (Figure 3).

Tubuloglomerular feedback is tonically active and maintains MD NaCl—and normally also GFR—at levels below those observed without the MD input. The operating point of the system defined as the steady-state GFR at steady-state MD NaCl concentration is located within the steepest part of tubuloglomerular feedback function curve (63). Thus, both increases and decreases in MD NaCl will cause tubuloglomerular feedback-mediated adjustments of afferent arteriolar resistance. The power of tubuloglomerular feedback to compensate for a flow perturbation is maximal for small changes around the operating point of the system (64,65). An acute change in arterial blood pressure unveils the regulatory effect of tubuloglomerular feedback. Both increases and decreases in arterial pressure elicit parallel adjustments in renal arterial resistance that mainly reflect that of afferent arterioles. These autoregulatory adjustments are impaired in the absence of tubuloglomerular feedback, suggesting that MD input is required to maintain renal blood flow during changes in arterial pressure (66,67). In preventing arterial pressure from affecting blood flow and GFR, tubuloglomerular feedback acts in cooperation with at least one additional mechanism, probably the myogenic property of all vascular smooth muscle cells, to respond to increasing luminal pressure with increasing resistance (68).

Tubuloglomerular feedback regulation of preglomerular resistance also creates a functional link between primary changes in PCT reabsorption and filtered load. Micropuncture studies in NHE3- or AQP1-deficient mice have documented

unchanged delivery of fluid into the DCT despite the expected major reductions in PCT fluid reabsorption (69,70). Normalization of distal delivery in these cases was due entirely to a reduction of GFR. The cause for the GFR reduction was tubuloglomerular feedback activation because it was not seen when tubuloglomerular feedback was interrupted. Similar correlations between inhibition of PCT fluid transport and GFR have been observed in a range of settings, including administration of carbonic anhydrase inhibitors, claudin2-deficient mice, mice with selective deletion of angiotensin II type 1 receptors for angiotensin II in the proximal tubule, and a mouse model of methyl malonic aciduria (11,71–73). The causes for the GFR reduction in some of these cases appear multifactorial. In addition to tubuloglomerular feedback, they may include effects of elevated tubular pressure and of the constrictor consequences of extracellular volume depletion. Nevertheless, the decrease in GFR is the major reason why catastrophic salt losses are usually not encountered with major failures of proximal reabsorption. The correlation between GFR and proximal reabsorption also holds during primary increments of PCT reabsorption. For example, there is evidence to support the notion that the rise of GFR in rats fed a high-protein diet and in streptozotocin-induced diabetes mellitus is the consequence of tubuloglomerular feedback due to enhanced proximal nephron Na reabsorption (74,75).

Thick Ascending Limbs of the Loop of Henle

Much of the evidence for flow-dependent NaCl reabsorption along the loop of Henle is based on *in vivo* microperfusion studies of the entire loop, including the *pars recta*. Nevertheless, the furosemide sensitivity of NaCl reabsorption leaves little doubt that flow dependence of loop Na transport is mediated mainly by the TALH (15,76). A 4-fold increase in flow rate caused a doubling of NaCl reabsorption, indicating that, in contrast to the proximal tubule, fractional reabsorption of NaCl fell with increasing flow rates (15). Flow dependence of TALH NaCl transport results from dependence of NKCC2 activity on luminal NaCl concentration (77). At low flows, luminal NaCl decreases along the length of the TALH from an isotonic or hypertonic starting value to a minimum of about 30 mM at the exit of the cortical TALH. If flow increases (as a result

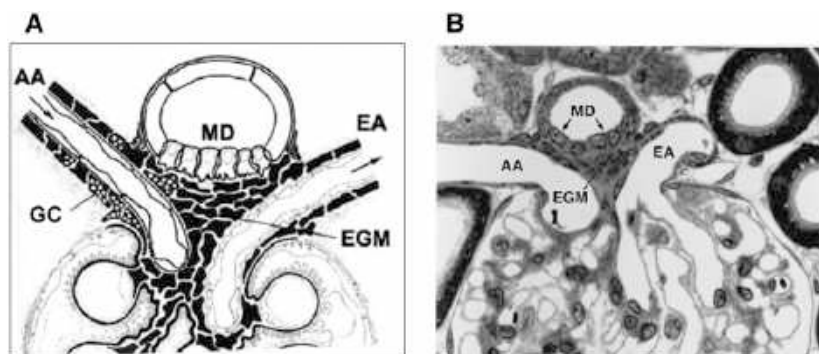


Figure 2. | The juxtaglomerular apparatus. Schematic (A) and low-power electron micrograph (B) (original magnification, $\times 320$) of the juxtaglomerular apparatus at the glomerular vascular pole. (Courtesy of B. Kaissling and W. Kriz). AA, afferent arteriole; EA, efferent arteriole; EGM, extraglomerular mesangium; GC granular cells; MD, macula densa.

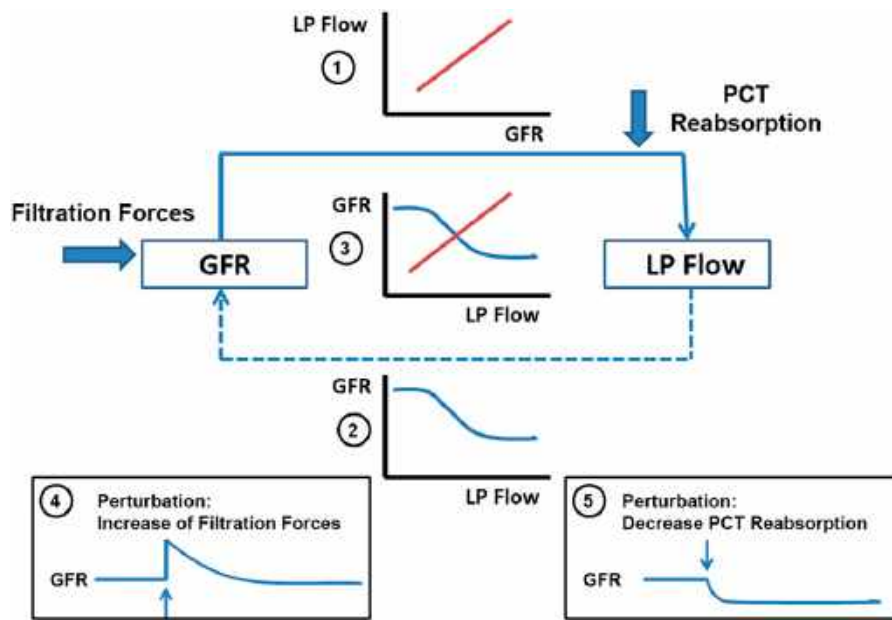


Figure 3. | Feedback relationship between GFR and proximal nephron Na reabsorption. While GFR directly determines Na reabsorption and thereby late proximal (LP) flow rate (solid arrow and inset 1), LP flow rate inversely affects GFR (broken arrow and inset 2). The intersection of the two relationships when plotted in the same graph (inset 3) defines the operating point of this feedback system. LP flow rate is an experimentally controllable surrogate of NaCl concentration at the macula densa sensor site. Bold arrows indicate two principal perturbations: a change in filtration forces and a change in Na reabsorption. Insets 4 and 5 indicate that GFR will tend to return to normal with changes in the former but deviate from normal with changes in the latter.

of GFR increases or proximal transport decreases), the fall in NaCl concentration is slowed, and the higher luminal concentrations enhance NaCl transport. Under these conditions, minimum concentrations are not attained, resulting in flow-dependent increases in NaCl concentration at the TALH exit (15,76,77,61).

TALH cells release several inhibitory mediators in response to increased flow that may partially counteract these effects. For example, an increase in shear stress stimulates the release of ATP, probably mediated by the central cilium (78). Luminal ATP increases cytoplasmic Ca^{2+} concentration ($[\text{Ca}]_i$) through activation of apical P2Y receptors (78,79). $[\text{Ca}]_i$ then activates NOS3, leading to flow-dependent release of nitric oxide (80). Increased $[\text{Ca}]_i$ also activates phospholipase A_2 and causes the formation of 20-HETE, another inhibitor of TALH NaCl transport (81). The release of these agents may explain why relative changes of TALH NaCl transport are less than those in delivery.

Distal Convoluted Tubule

Although the physiologic significance is less well understood, Na transport in distal tubular segments also depends on rates of Na and/or fluid delivery. *In vivo* microperfusion measurements (82) indicated a 4-fold increase in net Na transport by the rat DCT when perfused with solutions containing 148 mM Na (similar to plasma) compared with 75 mM Na (similar to distal tubular fluid *in vivo*). The effect presumably involves NCC because it was largest in the early part of the DCT. The mechanism behind this effect is not clear. It does not seem to involve a simple substrate activation of transport as the measured apparent K_m values of both Na and Cl for transport by NCC are <10 mM (83). It

is also not the result of maintaining luminal Na along the nephron as there were only small differences between perfused and collected fluid. It therefore appears to be a regulatory process involving luminal or intracellular Na or Cl concentrations as the perfusion rates were constant and shear stress did not change.

Cortical Collecting Duct

In vitro perfusion of isolated rabbit CCD showed that Na transport, mediated by ENaC, increases with perfusion rate over the physiologic range (84,85). In this case, the Na concentration remained constant as the volume flow increased. Again, a simple substrate effect can be ruled out, indicating regulation of the system. Here the most likely signal is mechanical. In a heterologous expression system, the channels were activated by shear stress on the membrane (86). It is also possible that increased flow dilutes an inhibitory factor such as ATP that might be secreted into the tubular lumen (87,88).

Inner Medullary Collecting Duct

As assessed by *in vivo* microcatheterization of the IMCD, rates of Na^+ reabsorption were proportional to rates of Na delivery to the segment and modified by infusion of KCl (43). This phenomenon was not observed in isolated perfused tubules (37), but in that preparation high flow rates abolish the effect of atrial natriuretic peptide (ANP) to inhibit absorption.

Chronic Effects

In addition to the acute effects described above, chronic changes in Na delivery from upstream segments can

further modulate downstream transport systems. Treatment of animals with furosemide inhibits Na reabsorption in the TALH and increases delivery to the distal nephron. This increases the reabsorptive capacity of the DCT (17,89) and is accompanied by ultrastructural changes in DCT cells (17,90), including increased cell volume (hypertrophy), increased luminal and basolateral surface area, and mitochondrial density. The remodeling of the cells continued in the CNT and CCD (91), implying that these segments were also affected by chronic increases in delivery rates. These changes attenuate the effects of the diuretic.

The signaling pathways involved in these events are unknown, but it is possible that increased intracellular Na^+ (or Cl^-) contributes to the phenomena. DCT cells also undergo hypertrophy in animal models of familial hyperkalemia with hypertension (19) that involve primary upregulation of NCC (92,93), thus increasing NaCl entry into the epithelial cells. Conversely, in NCC-knockout mice, the DCT cells undergo atrophy (94). In the rat CCD, mineralocorticoid infusion also produces hypertrophy (95) associated with increased Na/K-ATPase activity (96). Activation of the pump was prevented by blocking Na channels with amiloride, suggesting that increased Na entry into the cells was required for this effect.

Effects of Dietary Na and Extracellular Volume

While the responses to flow and Na delivery tend to keep Na outflow constant, matching Na excretion to Na intake requires that the kidneys produce urine with highly variable Na content. Na intake as determined by 24-hour urinary Na excretion varies in different populations from <1 mmol/d to 250 mmol/d (97). Changes in intake and body Na content are sensed through alterations in effective arterial blood volume and its effect on pressure-sensitive receptors in the vascular wall, the renal afferent arteriole, and the heart. The activation state of these receptors leads to changes in renal effector systems, such as the renin-angiotensin II-aldosterone axis, the renal sympathetic nervous system, and the release of vasopressin and ANP.

The neural and hormonal effector systems regulated by extracellular fluid volume cause changes of Na excretion mainly by changes in tubular Na reabsorption, especially in the distal nephron (Figure 4). Even with extreme variations in dietary intake, changes in fractional reabsorption are not very large because of the excess amounts of Na filtered under all circumstances. Renal fractional Na reabsorption with plausible 10-fold changes in Na intake vary only between approximately 97% and 99.7%. Thus, even with extreme intake variations, Na reabsorption fluctuates by only about 3% of filtered Na. Because of the gross imbalance between filtered and excreted Na it is not easy to exclude a role of a small change in GFR in the altered rate of Na excretion.

Proximal Nephron

Most evidence suggests that the role of the proximal tubule in the response to changes in Na intake is small. Typically, variations in NaCl intake are not associated with consistent and reproducible changes in GFR or proximal fluid reabsorption (98,99). Accordingly, Na delivery to the early DCT as assessed by micropuncture does not change dramatically with Na intake (17,18) (Figure 4).

The limited role of the PCT in the regulation of the excretion of Na following changes in dietary intake is puzzling because some of the effector systems responding to effective circulating volume (ECV) have clear effects on NaCl reabsorption in the PCT. Activation of the renal sympathetic nervous system stimulates Na transport through α_1 receptors, while renal denervation can reduce rates of Na reabsorption by 40% (100,101). During anesthesia, these changes were not accompanied by measurable alterations in renal hemodynamics, but this is less clear in conscious animals (101). In addition, angiotensin II, in a physiologic dose range, appears to stimulate NaHCO_3 reabsorption through activation of NHE3 and to enhance fluid reabsorption through its effect on filtration fraction and peritubular oncotic pressure (102–104). Thus, a reduction of ECV with stimulation of the renal nervous system and renin-angiotensin system should lead to stimulation of Na transport in the PCT. Furthermore, dopamine produced in the PCT is known to inhibit both Na,K-ATPase and NHE3 (105,106). Because dopamine synthesis is enhanced in ECV expansion, this would be another mediator that should contribute to the regulation of PCT NaCl transport with changes in dietary salt content (106). The reasons for the apparent neutral net effects of these potent mediators on the regulation of PT absorption by dietary salt intake are complex and will be difficult to untangle. Intra- and intersegmental compensation is likely to occur with blunting of early proximal events by downstream segments. Furthermore, possible interactions between these different agents and between them and other potential transport modulators (such as endothelin, nitric oxide, 20-HETE, cardiotonic steroids, and nucleotides/nucleosides) could make the final outcome, with respect to net effects on Na transport in the complete system, highly unpredictable. Finally, results from *in vitro* experimental models could lead to conclusions that do not apply to *in vivo* conditions.

More dramatic changes in extracellular fluid volume, such as those experimentally induced by the infusion of large fluid volumes, increase GFR and do indeed often reduce fractional fluid reabsorption (107). These factors combine to cause marked elevations in fluid delivery to the loops of Henle. Furthermore, long-term activation or inhibition of angiotensin II-mediated transport in the proximal tubule can affect BP, presumably through progressive changes of body Na content (71,108).

Distal Convoluted Tubule

In vivo micropuncture measurements in rat kidney showed that Na reabsorption in the early DCT doubled under chronic conditions when dietary Na intake was reduced for >1 week (17). This was accompanied by a similar increase in Cl reabsorption and was blocked by thiazides, indicating an effect on NCC-mediated transport (Figure 5). Biochemical measurements on rat kidney indicated an increase in the surface expression of this transporter in Na-depleted animals (109). The signals mediating this response have not been established. The renin-angiotensin-aldosterone (RAA) axis is stimulated under these conditions, and long-term infusion of aldosterone increased NCC expression in some (110,111), but not all (109,112), studies. However, mineralocorticoids may not be the major regulators of NCC because a high-K diet increases

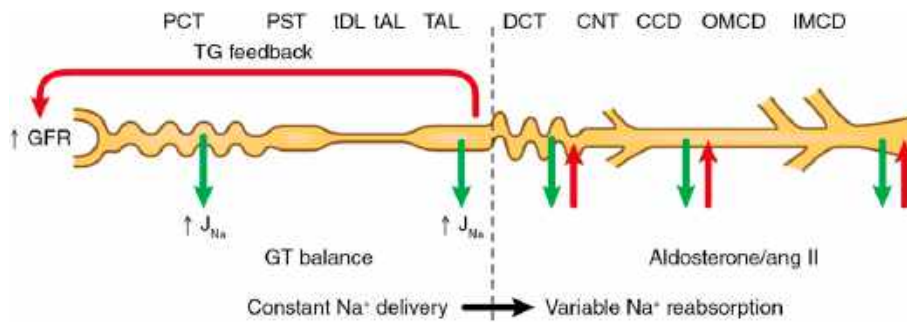


Figure 4. | Effects of glomerulotubular (GT) balance and tubuloglomerular (TG) feedback on delivery of Na to the distal nephron. These feedback loops minimize changes in GFR and in the amount of Na escaping the TALH. Changes in Na reabsorption in the distal nephron, including the DCT, CNT, and collecting duct, effect variations in Na excretion. ang II, angiotensin II; CCD, cortical collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; J_{Na} , sodium flux; OMCD, outer medullary collecting duct; PCT, proximal convoluted tubule; PST, proximal straight tubule; tAL, thin ascending limb; TAL, thick ascending limb of Henle's loop; tDL, thin descending limb.

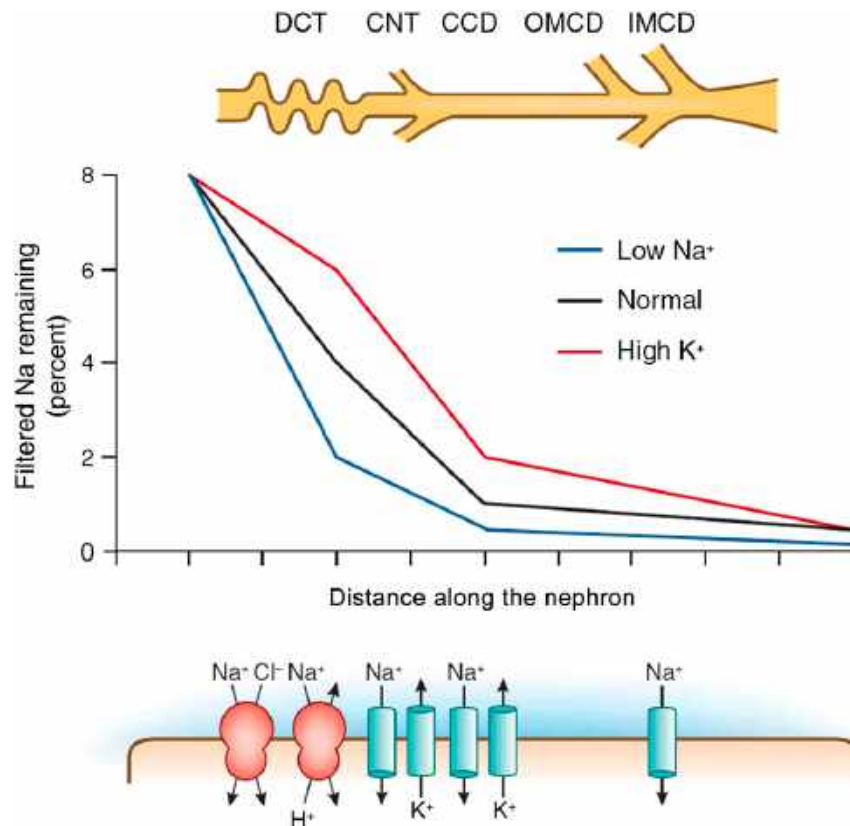


Figure 5. | Variations in the profile of Na transport during dietary Na restriction (blue line) and dietary K loading (red line). With Na restriction, transport by all distal segments is stimulated. With K loading, Na channel–mediated transport in the CNT and collecting duct, which effectively exchanges Na for K, is augmented, while NaCl cotransport in the DCT is diminished. CCD, cortical collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; IMCD, inner medullary collecting duct; OMCD, outer medullary collecting duct.

aldosterone secretion but decreases cotransporter activity. Angiotensin II is another candidate for upregulating NCC. In microperfusion experiments, application of angiotensin II increased DCT Na transport by both early and late DCT segments, suggesting upregulation of Na/H exchange and Na channels, respectively (21). Acute changes in the hormone level *in vivo* led to redistribution of NCC between the cell surface and intracellular vesicles of DCT cells (113).

Long-term infusion of this peptide increases NCC expression in rat kidney (114).

CNT/CCD

Dietary Na depletion strongly increases Na transport by the CCD measured *in vitro* (28,29,115) (Figure 5). Na channel activity rises in parallel (30), consistent with upregulation of ENaC as the major event in this process. Electrophysiologic

measurements indicated similar responses in the CNT (25) and in the late DCT (DCT2) (26). In the latter case, the dependence of the channels on dietary salt was shifted with activity appearing at higher levels of Na intake.

The increased Na channel activity observed in the CCD when animals are Na-depleted can be quantitatively mimicked by treatment with aldosterone (30), suggesting that increases in this hormone account for the chronic response of this segment. Indeed, these segments are collectively referred to as the aldosterone-sensitive distal nephron (ASDN). However angiotensin II acutely increased ENaC activity (116). Furthermore, chronic angiotensin II elevation can also increase ENaC activity independent of aldosterone (117), indicating separate effects of these two arms of the RAA axis on channel-mediated Na reabsorption.

Dietary Na depletion also upregulates the bicarbonate-dependent NaCl transporter in the CCD (32). This system also appears to be inhibited by ANP (118), a hormone released from the heart in response to expansion of plasma volume (119).

Inner Medullary Collecting Duct

Split-drop experiments in the IMCD indicated that this segment could also participate in the response to dietary Na depletion (38). Transport was stimulated modestly by aldosterone and inhibited by amiloride, consistent with an upregulation of ENaC as in the CCD. This idea is supported by biochemical measurements showing increased abundance of α - and β ENaC and increased proteolytic cleavage of α - and γ ENaC in the inner medulla of the rat kidney (33). ANP decreased amiloride-sensitive O_2 consumption in isolated IMCD cells (120) and net Na reabsorption in IMCD measured *in vivo* (43) or *in vitro* (37).

Effects of Dietary K

Changes in dietary K intake also affect Na transport along the nephron. If this occurs with constant Na intake, achieving Na balance demands that K excretion must vary without altering net Na excretion. In this case, the distribution of Na reabsorption along the nephron shifts between more proximal segments (where the K is reabsorbed with Na) and the CNT/CCD (where K is secreted by principal cells in exchange for Na [121]) (Figure 5).

Acute increases in plasma K are natriuretic (122,123). This effect may reflect reduced Na^+ reabsorption in the proximal tubule (124), the TALH (125), or the DCT (126). As argued earlier, perturbations in the more proximal segments tend to be largely compensated by GTB and tubuloglomerular feedback. However, inhibition of reabsorption in the DCT will directly increase delivery of Na^+ to the ASDN, stimulating both Na^+ reabsorption and K^+ secretion. Acute K loading leads to a decrease in the phosphorylation of the thiazide-sensitive cotransporter NCC (126), consistent with reduced activity (19).

Under more chronic conditions, the total amount of NCC as well as phospho-NCC in the cell (127) decreases as K intake increases. The surface expression of NCC also changes reciprocally with dietary K (128). At the same time, the activity of ENaC in the CCD (129) and CNT (25,130) rises with the K load, together with parallel upregulation of apical K^+ channels (129,131) and the Na,K-ATPase (129). These

responses will shift Na^+ transport from the DCT, where Na^+ is reabsorbed together with Cl^- , to the ASDN, where K^+ secretion balances, at least in part, the transport of Na^+ . In this way both Na and K can remain in balance. Achievement of Na balance in the presence of elevated aldosterone levels has been called “aldosterone escape.”

The opposite shift will occur when dietary K intake is restricted: NCC surface expression and presumably activity increase, limiting Na delivery to downstream K-secreting segments. This response is exaggerated with simultaneous Na and K depletion (132). Here, ENaC activity is suppressed, despite low salt intake. NCC abundance increases strongly, indicating that much of the burden of Na retention falls on the DCT under these conditions.

The RAA axis plays an important role in shifting Na reabsorption from one segment to the other. K loading increases aldosterone production independent of angiotensin II, contributing to the upregulation of ENaC, although an aldosterone-independent pathway may also be important (129). The identities of signals regulating NCC are uncertain. Angiotensin II can stimulate NCC expression independent of aldosterone (114), and it is possible that this hormone may mediate, at least in part, the effects of changes in dietary K. A low-K diet increases plasma renin activity (133), and angiotensin II levels are likely to change reciprocally with dietary K intake.

Acknowledgments

L.G.P. acknowledges the support of Grant R01-DK27847 from the National Institutes of Health. J.S. was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Disclosures

None.

References

1. U.S. Department of Agriculture, Agricultural Research Service. Nutrient intakes from food: Mean amounts consumed per individual, by gender and age. In: What We Eat In America, NHANES 2009-2010, 2012. Available at: http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/0708/Table_1_NIN_GEN_07.pdf. Accessed June 25, 2014
2. Titze J: Sodium balance is not just a renal affair. *Curr Opin Nephrol Hypertens* 23: 101–105, 2014
3. Reinhardt HW, Behrenbeck DW: [Studies in awake dogs on the regulation of the sodium balance. I. The significance of the extracellular space for the regulation of the daily sodium balance]. *Pflügers Arch Gesamte Physiol Menschen Tiere* 295: 266–279, 1967
4. He FJ, Li J, Macgregor GA: Effect of longer-term modest salt reduction on blood pressure. *Cochrane Database Syst Rev* 4: CD004937, 2013
5. Liu FY, Cogan MG: Axial heterogeneity of bicarbonate, chloride, and water transport in the rat proximal convoluted tubule. Effects of change in luminal flow rate and of alkalemia. *J Clin Invest* 78: 1547–1557, 1986
6. Curthoys NP, Moe OW: Proximal tubule function and response to acidosis [published online ahead of print May 1, 2014]. *Clin J Am Soc Nephrol* doi: 10.2215/CJN.10391012
7. Frömter E, Rumrich G, Ullrich KJ: Phenomenologic description of Na^+ , Cl^- and HCO_3^- absorption from proximal tubules of rat kidney. *Pflügers Arch* 343: 189–220, 1973
8. Amasheh S, Meiri N, Gitter AH, Schöneberg T, Mankertz J, Schulzke JD, Fromm M: Claudin-2 expression induces

- cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 115: 4969–4976, 2002
9. Muto S, Hata M, Taniguchi J, Tsuruoka S, Moriwaki K, Saitou M, Furuse K, Sasaki H, Fujimura A, Imai M, Kusano E, Tsukita S, Furuse M: Claudin-2-deficient mice are defective in the leaky and cation-selective paracellular permeability properties of renal proximal tubules. *Proc Natl Acad Sci U S A* 107: 8011–8016, 2010
 10. Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, Günzel D, Fromm M: Claudin-2, a component of the tight junction, forms a paracellular water channel. *J Cell Sci* 123: 1913–1921, 2010
 11. Schnermann J, Huang Y, Mizel D: Fluid reabsorption in proximal convoluted tubules of mice with gene deletions of claudin-2 and/or aquaporin1. *Am J Physiol Renal Physiol* 305: F1352–F1364, 2013
 12. Sands JM, Layton HE: The urine concentrating mechanism and urea transporters. In: *The Kidney Physiology and Pathophysiology*, edited by Alpern RJ, Caplan MJ, Moe OW, 5th Ed., London, Waltham, San Diego, Elsevier Academic Press, 2013, pp 1463–1510
 13. Mount DB: Renal physiology: thick ascending limb. *Clin J Am Soc Nephrol* 2014, in press
 14. Schnermann J, Castrop H: Function of the juxtaglomerular apparatus: control of glomerular hemodynamics and renin secretion. In: *The Kidney. Physiology and Pathophysiology*, edited by Alpern RJ, Caplan MJ, Moe OW, 5th Ed., London, Waltham, San Diego, Elsevier Academic Press, 2013, pp 757–801
 15. Wright FS, Schnermann J: Interference with feedback control of glomerular filtration rate by furosemide, triflocin, and cyanide. *J Clin Invest* 53: 1695–1708, 1974
 16. Costanzo LS: Comparison of calcium and sodium transport in early and late rat distal tubules: effect of amiloride. *Am J Physiol* 246: F937–F945, 1984
 17. Ellison DH, Velázquez H, Wright FS: Adaptation of the distal convoluted tubule of the rat. Structural and functional effects of dietary salt intake and chronic diuretic infusion. *J Clin Invest* 83: 113–126, 1989
 18. Malnic G, Klose RM, Giebisch G: Micropuncture study of distal tubular potassium and sodium transport in rat nephron. *Am J Physiol* 211: 529–547, 1966
 19. Subramanya AR, Ellison DH: Distal convoluted tubule [Published online ahead of print May 22, 2014]. *Clin J Am Soc Nephrol* doi: 10.2215/CJN.0592061
 20. Wang T, Hropot M, Aronson PS, Giebisch G: Role of NHE isoforms in mediating bicarbonate reabsorption along the nephron. *Am J Physiol Renal Physiol* 281: F1117–F1122, 2001
 21. Wang T, Giebisch G: Effects of angiotensin II on electrolyte transport in the early and late distal tubule in rat kidney. *Am J Physiol* 271: F143–F149, 1996
 22. Wang T, Malnic G, Giebisch G, Chan YL: Renal bicarbonate reabsorption in the rat. IV. Bicarbonate transport mechanisms in the early and late distal tubule. *J Clin Invest* 91: 2776–2784, 1993
 23. Almeida AJ, Burg MB: Sodium transport in the rabbit connecting tubule. *Am J Physiol* 243: F330–F334, 1982
 24. Shareghi GR, Stoner LC: Calcium transport across segments of the rabbit distal nephron in vitro. *Am J Physiol* 235: F367–F375, 1978
 25. Frindt G, Palmer LG: Na channels in the rat connecting tubule. *Am J Physiol Renal Physiol* 286: F669–F674, 2004
 26. Nesterov V, Dahlmann A, Krueger B, Bertog M, Löffing J, Korbmayer C: Aldosterone-dependent and -independent regulation of the epithelial sodium channel (ENaC) in mouse distal nephron. *Am J Physiol Renal Physiol* 303: F1289–F1299, 2012
 27. Löffing J, Pietri L, Aregger F, Bloch-Faure M, Ziegler U, Meneton P, Rossier BC, Kaissling B: Differential subcellular localization of ENaC subunits in mouse kidney in response to high- and low-Na diets. *Am J Physiol Renal Physiol* 279: F252–F258, 2000
 28. Reif MC, Troutman SL, Schafer JA: Sodium transport by rat cortical collecting tubule. Effects of vasopressin and desoxycorticosterone. *J Clin Invest* 77: 1291–1298, 1986
 29. Tomita K, Pisano JJ, Knepper MA: Control of sodium and potassium transport in the cortical collecting duct of the rat. Effects of bradykinin, vasopressin, and deoxycorticosterone. *J Clin Invest* 76: 132–136, 1985
 30. Pácha J, Frindt G, Antonian L, Silver RB, Palmer LG: Regulation of Na channels of the rat cortical collecting tubule by aldosterone. *J Gen Physiol* 102: 25–42, 1993
 31. Palmer LG, Frindt G: Amiloride-sensitive Na channels from the apical membrane of the rat cortical collecting tubule. *Proc Natl Acad Sci U S A* 83: 2767–2770, 1986
 32. Levieil F, Hübner CA, Houillier P, Morla L, El Moghrabi S, Brideau G, Hassan H, Parker MD, Kurth I, Kougiumtzes A, Sinning A, Pech V, Riemyndy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, Eladari D: The Na⁺-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na⁺ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest* 120: 1627–1635, 2010
 33. Frindt G, Ergonul Z, Palmer LG: Na channel expression and activity in the medullary collecting duct of rat kidney. *Am J Physiol Renal Physiol* 292: F1190–F1196, 2007
 34. Stokes JB, Ingram MJ, Williams AD, Ingram D: Heterogeneity of the rabbit collecting tubule: Localization of mineralocorticoid hormone action to the cortical portion. *Kidney Int* 20: 340–347, 1981
 35. Sands JM, Nonoguchi H, Knepper MA: Hormone effects on NaCl permeability of rat inner medullary collecting duct. *Am J Physiol* 255: F421–F428, 1988
 36. Kudo LH, van Baak AA, Rocha AS: Effect of vasopressin on sodium transport across inner medullary collecting duct. *Am J Physiol* 258: F1438–F1447, 1990
 37. Rocha AS, Kudo LH: Atrial peptide and cGMP effects on NaCl transport in inner medullary collecting duct. *Am J Physiol* 259: F258–F268, 1990
 38. Ullrich KJ, Papavassiliou F: Sodium reabsorption in the papillary collecting duct of rats. Effect of adrenalectomy, low Na⁺ diet, acetazolamide, HCO₃⁻-free solutions and of amiloride. *Pflügers Arch* 379: 49–52, 1979
 39. Sonnenberg H, Honrath U, Wilson DR: Effects of amiloride in the medullary collecting duct of rat kidney. *Kidney Int* 31: 1121–1125, 1987
 40. Mancilha-Carvalho JJ, de Oliveira R, Esposito RJ: Blood pressure and electrolyte excretion in the Yanomamo Indians, an isolated population. *J Hum Hypertens* 3: 309–314, 1989
 41. Grantham JJ, Kurg MB, Obloff J: The nature of transtubular Na and K transport in isolated rabbit renal collecting tubules. *J Clin Invest* 49: 1815–1826, 1970
 42. Imai M, Yoshitomi K: Electrophysiological study of inner medullary collecting duct of hamsters. *Pflügers Arch* 416: 180–188, 1990
 43. Sonnenberg H, Honrath U, Chong CK, Wilson DR: Atrial natriuretic factor inhibits sodium transport in medullary collecting duct. *Am J Physiol* 250: F963–F966, 1986
 44. Erlj D: Basic electrical properties of tight epithelia determined with a simple method. *Pflügers Arch* 364: 91–93, 1976
 45. Higgins JT Jr, Gebler B, Frömter E: Electrical properties of amphibian urinary bladder epithelia. II. The cell potential profile in *necturus maculosus*. *Pflügers Arch* 371: 87–97, 1977
 46. Lewis SA, Eaton DC, Diamond JM: The mechanism of Na⁺ transport by rabbit urinary bladder. *J Membr Biol* 28: 41–70, 1976
 47. Smith HW: *The Kidney. Structure and Function in Health and Disease*, New York, Oxford University Press, 1951
 48. Landwehr DM, Schnermann J, Klose RM, Giebisch G: Effect of reduction in filtration rate on renal tubular sodium and water reabsorption. *Am J Physiol* 215: 687–695, 1968
 49. Lindheimer MD, Lalone RC, Levinsky NG: Evidence that an acute increase in glomerular filtration has little effect on sodium excretion in the dog unless extracellular volume is expanded. *J Clin Invest* 46: 256–265, 1967
 50. Glabman S, Aynedjian HS, Bank N: Micropuncture study of the effect of acute reductions in glomerular filtration rate on sodium and water reabsorption by the proximal tubules of the rat. *J Clin Invest* 44: 1410–1416, 1965
 51. Schnermann J, Wahl M, Liebau G, Fischbach H: Balance between tubular flow rate and net fluid reabsorption in the proximal convolution of the rat kidney. I. Dependency of reabsorptive net

- fluid flux upon proximal tubular surface area at spontaneous variations of filtration rate. *Pflügers Arch* 304: 90–103, 1968
52. Brenner BM, Troy JL: Postglomerular vascular protein concentration: Evidence for a causal role in governing fluid reabsorption and glomerulotubular balance by the renal proximal tubule. *J Clin Invest* 50: 336–349, 1971
 53. Brenner BM, Troy JL, Daugharty TM: On the mechanism of inhibition in fluid reabsorption by the renal proximal tubule of the volume-expanded rat. *J Clin Invest* 50: 1596–1602, 1971
 54. Falchuk KH, Brenner BM, Tadokoro M, Berliner RW: Oncotic and hydrostatic pressures in peritubular capillaries and fluid reabsorption by proximal tubule. *Am J Physiol* 220: 1427–1433, 1971
 55. Lewy JE, Windhager EE: Peritubular control of proximal tubular fluid reabsorption in the rat kidney. *Am J Physiol* 214: 943–954, 1968
 56. Barfuss DW, Schafer JA: Flow dependence of nonelectrolyte absorption in the nephron. *Am J Physiol* 236: F163–F174, 1979
 57. Burg M, Patlak C, Green N, Villey D: Organic solutes in fluid absorption by renal proximal convoluted tubules. *Am J Physiol* 231: 627–637, 1976
 58. Bartoli E, Conger JD, Earley LE: Effect of intraluminal flow on proximal tubular reabsorption. *J Clin Invest* 52: 843–849, 1973
 59. Du Z, Duan Y, Yan Q, Weinstein AM, Weinbaum S, Wang T: Mechanosensory function of microvilli of the kidney proximal tubule. *Proc Natl Acad Sci U S A* 101: 13068–13073, 2004
 60. Du Z, Yan Q, Duan Y, Weinbaum S, Weinstein AM, Wang T: Axial flow modulates proximal tubule NHE3 and H-ATPase activities by changing microvillus bending moments. *Am J Physiol Renal Physiol* 290: F289–F296, 2006
 61. Wright FS: Flow-dependent transport processes: Filtration, absorption, secretion. *Am J Physiol* 243: F1–F11, 1982
 62. Briggs J: A simple steady-state model for feedback control of glomerular filtration rate. *Kidney Int Suppl* 12[Suppl. 12]: S143–S150, 1982
 63. Briggs JP, Schubert G, Schnermann J: Quantitative characterization of the tubuloglomerular feedback response: effect of growth. *Am J Physiol* 247: F808–F815, 1984
 64. Moore LC, Mason J: Perturbation analysis of tubuloglomerular feedback in hydropenic and hemorrhaged rats. *Am J Physiol* 245: F554–F563, 1983
 65. Thomson SC, Blantz RC: Homeostatic efficiency of tubuloglomerular feedback in hydropenia, euvoolemia, and acute volume expansion. *Am J Physiol* 264: F930–F936, 1993
 66. Moore LC, Schnermann J, Yarimizu S: Feedback mediation of SNGFR autoregulation in hydropenic and DOCA- and salt-loaded rats. *Am J Physiol* 237: F63–F74, 1979
 67. Navar LG, Burke TJ, Robinson RR, Clapp JR: Distal tubular feedback in the autoregulation of single nephron glomerular filtration rate. *J Clin Invest* 53: 516–525, 1974
 68. Holstein-Rathlou NH, Wagner AJ, Marsh DJ: Tubuloglomerular feedback dynamics and renal blood flow autoregulation in rats. *Am J Physiol* 260: F53–F68, 1991
 69. Lorenz JN, Schultheis PJ, Traynor T, Shull GE, Schnermann J: Micropuncture analysis of single-nephron function in NHE3-deficient mice. *Am J Physiol* 277: F447–F453, 1999
 70. Schnermann J, Chou C-L, Ma T, Traynor T, Knepper MA, Verkman AS: Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci U S A* 95: 9660–9664, 1998
 71. Gurley SB, Riquier-Brison AD, Schnermann J, Sparks MA, Allen AM, Haase VH, Snouwaert JN, Le TH, McDonough AA, Koller BH, Coffman TM: AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab* 13: 469–475, 2011
 72. Manoli I, Sysol JR, Li L, Houillier P, Garone C, Wang C, Zerfas PM, Cusmano-Ozog K, Young S, Trivedi NS, Cheng J, Sloan JL, Chandler RJ, Abu-Asab M, Tsokos M, Elkahlon AG, Rosen S, Enns GM, Berry GT, Hoffmann V, DiMauro S, Schnermann J, Venditti CP: Targeting proximal tubule mitochondrial dysfunction attenuates the renal disease of methylmalonic acidemia. *Proc Natl Acad Sci U S A* 110: 13552–13557, 2013
 73. Tucker BJ, Steiner RW, Gushwa LC, Blantz RC: Studies on the tubulo-glomerular feedback system in the rat. The mechanism of reduction in filtration rate with benzolamide. *J Clin Invest* 62: 993–1004, 1978
 74. Seney FD Jr, Persson EG, Wright FS: Modification of tubuloglomerular feedback signal by dietary protein. *Am J Physiol* 252: F83–F90, 1987
 75. Thomson SC, Deng A, Komine N, Hammes JS, Blantz RC, Gabbai FB: Early diabetes as a model for testing the regulation of juxtaglomerular NOS I. *Am J Physiol Renal Physiol* 287: F732–F738, 2004
 76. Morgan T, Berliner RW: A study by continuous microperfusion of water and electrolyte movements in the loop of Henle and distal tubule of the rat. *Nephron* 6: 388–405, 1969
 77. Greger R: Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol Rev* 65: 760–797, 1985
 78. Jensen ME, Odgaard E, Christensen MH, Praetorius HA, Leipziger J: Flow-induced $[Ca^{2+}]_i$ increase depends on nucleotide release and subsequent purinergic signaling in the intact nephron. *J Am Soc Nephrol* 18: 2062–2070, 2007
 79. Praetorius HA, Leipziger J: Primary cilium-dependent sensing of urinary flow and paracrine purinergic signaling. *Semin Cell Dev Biol* 24: 3–10, 2013
 80. Cabral PD, Hong NJ, Garvin JL: ATP mediates flow-induced NO production in thick ascending limbs. *Am J Physiol Renal Physiol* 303: F194–F200, 2012
 81. Wang W, Lu M, Balazy M, Hebert SC: Phospholipase A2 is involved in mediating the effect of extracellular Ca^{2+} on apical K^+ channels in rat TAL. *Am J Physiol* 273: F421–F429, 1997
 82. Ellison DH, Velázquez H, Wright FS: Thiazide-sensitive sodium chloride cotransport in early distal tubule. *Am J Physiol* 253: F546–F554, 1987
 83. Monroy A, Plata C, Hebert SC, Gamba G: Characterization of the thiazide-sensitive Na^{+} - Cl^{-} cotransporter: A new model for ions and diuretics interaction. *Am J Physiol Renal Physiol* 279: F161–F169, 2000
 84. Engbretson BG, Stoner LC: Flow-dependent potassium secretion by rabbit cortical collecting tubule in vitro. *Am J Physiol* 253: F896–F903, 1987
 85. Woda CB, Bragin A, Kleyman TR, Satlin LM: Flow-dependent K^{+} secretion in the cortical collecting duct is mediated by a maxi-K channel. *Am J Physiol Renal Physiol* 280: F786–F793, 2001
 86. Carattino MD, Sheng S, Kleyman TR: Epithelial Na^{+} channels are activated by laminar shear stress. *J Biol Chem* 279: 4120–4126, 2004
 87. Ma HP, Li L, Zhou ZH, Eaton DC, Warnock DG: ATP masks stretch activation of epithelial sodium channels in A6 distal nephron cells. *Am J Physiol Renal Physiol* 282: F501–F505, 2002
 88. Pochynyuk O, Bugaj V, Vandewalle A, Stockand JD: Purinergic control of apical plasma membrane $PI(4,5)P_2$ levels sets ENaC activity in principal cells. *Am J Physiol Renal Physiol* 294: F38–F46, 2008
 89. Stanton BA, Kaissling B: Regulation of renal ion transport and cell growth by sodium. *Am J Physiol* 257: F1–F10, 1989
 90. Kaissling B, Stanton BA: Adaptation of distal tubule and collecting duct to increased sodium delivery. I. Ultrastructure. *Am J Physiol* 255: F1256–F1268, 1988
 91. Kaissling B, Le Hir M: Functional morphology of kidney tubules and cells in situ. *Methods Enzymol* 191: 265–289, 1990
 92. Lalioti MD, Zhang J, Volkman HM, Kahle KT, Hoffmann KE, Toka HR, Nelson-Williams C, Ellison DH, Flavell R, Booth CJ, Lu Y, Geller DS, Lifton RP: Wnk4 controls blood pressure and potassium homeostasis via regulation of mass and activity of the distal convoluted tubule. *Nat Genet* 38: 1124–1132, 2006
 93. Yang SS, Morimoto T, Rai T, Chiga M, Sahara E, Ohno M, Uchida K, Lin SH, Moriguchi T, Shibuya H, Kondo Y, Sasaki S, Uchida S: Molecular pathogenesis of pseudohypoaldosteronism type II: generation and analysis of a Wnk4(D561A/+ $^{-}$) knockin mouse model. *Cell Metab* 5: 331–344, 2007
 94. Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE: Phenotype resembling Gitelman's syndrome in mice lacking the apical Na^{+} - Cl^{-} cotransporter of the distal convoluted tubule. *J Biol Chem* 273: 29150–29155, 1998

95. Wade JB, Stanton BA, Field MJ, Kashgarian M, Giebisch G: Morphological and physiological responses to aldosterone: Time course and sodium dependence. *Am J Physiol* 259: F88–F94, 1990
96. Palmer LG, Antonian L, Frindt G: Regulation of the Na-K pump of the rat cortical collecting tubule by aldosterone. *J Gen Physiol* 102: 43–57, 1993
97. Intersalt Cooperative Research Group: Intersalt: An international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *BMJ* 297: 319–328, 1988
98. Schor N, Ichikawa I, Brenner BM: Glomerular adaptations to chronic dietary salt restriction or excess. *Am J Physiol* 238: F428–F436, 1980
99. Vallon V, Huang DY, Deng A, Richter K, Blantz RC, Thomson S: Salt-sensitivity of proximal reabsorption alters macula densa salt and explains the paradoxical effect of dietary salt on glomerular filtration rate in diabetes mellitus. *J Am Soc Nephrol* 13: 1865–1871, 2002
100. Bello-Reuss E, Colindres RE, Pastoriza-Muñoz E, Mueller RA, Gottschalk CW: Effects of acute unilateral renal denervation in the rat. *J Clin Invest* 56: 208–217, 1975
101. Johns EJ, Kopp UC: Neural control of renal function. In: *The Kidney Physiology and Pathophysiology*, edited by Alpern RJ, Caplan MJ, Moe OW, 5th Ed., London, Waltham, San Diego, Elsevier Academic Press, 2013, pp 451–486
102. Cogan MG: Angiotensin II: A powerful controller of sodium transport in the early proximal tubule. *Hypertension* 15: 451–458, 1990
103. Geibel J, Giebisch G, Boron WF: Angiotensin II stimulates both Na(+)-H+ exchange and Na+/HCO₃- cotransport in the rabbit proximal tubule. *Proc Natl Acad Sci U S A* 87: 7917–7920, 1990
104. Schuster VL, Kokko JP, Jacobson HR: Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. *J Clin Invest* 73: 507–515, 1984
105. Aperia A, Bertorello A, Seri I: Dopamine causes inhibition of Na+-K+-ATPase activity in rat proximal convoluted tubule segments. *Am J Physiol* 252: F39–F45, 1987
106. Jose PA, Felder RA, Eisner GM: Paracrine regulation of renal function by dopamine. In: *The Kidney Physiology and Pathophysiology*, edited by Alpern RJ, Caplan MJ, Moe OW, London, Waltham, San Diego, Elsevier Academic Press, 2013, pp 539–591
107. Osgood RW, Reineck HJ, Stein JH: Further studies on segmental sodium transport in the rat kidney during expansion of the extracellular fluid volume. *J Clin Invest* 62: 311–320, 1978
108. Li H, Weatherford ET, Davis DR, Keen HL, Grobe JL, Daugherty A, Cassis LA, Allen AM, Sigmund CD: Renal proximal tubule angiotensin AT1A receptors regulate blood pressure. *Am J Physiol Regul Integr Comp Physiol* 301: R1067–R1077, 2011
109. Frindt G, Palmer LG: Surface expression of sodium channels and transporters in rat kidney: effects of dietary sodium. *Am J Physiol Renal Physiol* 297: F1249–F1255, 2009
110. Kim GH, Masilamani S, Turner R, Mitchell C, Wade JB, Knepper MA: The thiazide-sensitive Na-Cl cotransporter is an aldosterone-induced protein. *Proc Natl Acad Sci U S A* 95: 14552–14557, 1998
111. van der Lubbe N, Lim CH, Meima ME, van Veghel R, Rosenbaek LL, Mutig K, Danser AH, Fenton RA, Zietse R, Hoorn EJ: Aldosterone does not require angiotensin II to activate NCC through a WNK4-SPAK-dependent pathway. *Pflugers Arch* 463: 853–863, 2012
112. Wang XY, Masilamani S, Nielsen J, Kwon TH, Brooks HL, Nielsen S, Knepper MA: The renal thiazide-sensitive Na-Cl cotransporter as mediator of the aldosterone-escape phenomenon. *J Clin Invest* 108: 215–222, 2001
113. Sandberg MB, Riquier AD, Pihakaski-Maunsbach K, McDonough AA, Maunsbach AB: Angiotensin II provokes acute trafficking of distal tubule NaCl cotransporter (NCC) to apical membrane. *Am J Physiol Renal Physiol* 293: F662–F669, 2007
114. van der Lubbe N, Lim CH, Fenton RA, Meima ME, Jan Danser AH, Zietse R, Hoorn EJ: Angiotensin II induces phosphorylation of the thiazide-sensitive sodium chloride cotransporter independent of aldosterone. *Kidney Int* 79: 66–76, 2011
115. Schwartz GJ, Burg MB: Mineralocorticoid effects on cation transport by cortical collecting tubules *in vitro*. *Am J Physiol* 235: F576–F585, 1978
116. Mamenko M, Zaika O, Ilatovskaya DV, Staruschenko A, Pochynyuk O: Angiotensin II increases activity of the epithelial Na+ channel (ENaC) in distal nephron additively to aldosterone. *J Biol Chem* 287: 660–671, 2012
117. Mamenko M, Zaika O, Prieto MC, Jensen VB, Doris PA, Navar LG, Pochynyuk O: Chronic angiotensin II infusion drives extensive aldosterone-independent epithelial Na+ channel activation. *Hypertension* 62: 1111–1122, 2013
118. Nonoguchi H, Sands JM, Knepper MA: ANF inhibits NaCl and fluid absorption in cortical collecting duct of rat kidney. *Am J Physiol* 256: F179–F186, 1989
119. Maack T: Role of atrial natriuretic factor in volume control. *Kidney Int* 49: 1732–1737, 1996
120. Zeidel ML, Seifter JL, Lear S, Brenner BM, Silva P: Atrial peptides inhibit oxygen consumption in kidney medullary collecting duct cells. *Am J Physiol* 251: F379–F383, 1986
121. Pearce D, Soundararajan R, Trimpert C, Kashlan OB, Deen PM, Kohan DE: Collecting duct principal cell transport processes and their regulation [published online ahead of print May 29, 2014]. *Clin J Am Soc Nephrol* doi: 10.2215/CJN.05760513
122. van Buren M, Rabelink TJ, van Rijn HJ, Koomans HA: Effects of acute NaCl, KCl and KHCO₃ loads on renal electrolyte excretion in humans. *Clin Sci (Lond)* 83: 567–574, 1992
123. Wright FS, Strieder N, Fowler NB, Giebisch G: Potassium secretion by distal tubule after potassium adaptation. *Am J Physiol* 221: 437–448, 1971
124. Brandis M, Keyes J, Windhager EE: Potassium-induced inhibition of proximal tubular fluid reabsorption in rats. *Am J Physiol* 222: 421–427, 1972
125. Stokes JB: Consequences of potassium recycling in the renal medulla. Effects of ion transport by the medullary thick ascending limb of Henle's loop. *J Clin Invest* 70: 219–229, 1982
126. Sorensen MV, Grossmann S, Roesinger M, Gresko N, Todkar AP, Barmettler G, Ziegler U, Odermatt A, Loffing-Cueni D, Loffing J: Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice. *Kidney Int* 83: 811–824, 2013
127. Vallon V, Schroth J, Lang F, Kuhl D, Uchida S: Expression and phosphorylation of the Na⁺-Cl⁻ cotransporter NCC *in vivo* is regulated by dietary salt, potassium, and SGK1. *Am J Physiol Renal Physiol* 297: F704–F712, 2009
128. Frindt G, Palmer LG: Effects of dietary K on cell-surface expression of renal ion channels and transporters. *Am J Physiol Renal Physiol* 299: F890–F897, 2010
129. Palmer LG, Antonian L, Frindt G: Regulation of apical K and Na channels and Na/K pumps in rat cortical collecting tubule by dietary K. *J Gen Physiol* 104: 693–710, 1994
130. Frindt G, Shah A, Edvinsson J, Palmer LG: Dietary K regulates ROMK channels in connecting tubule and cortical collecting duct of rat kidney. *Am J Physiol Renal Physiol* 296: F347–F354, 2009
131. Wang WH, Schwab A, Giebisch G: Regulation of small-conductance K+ channel in apical membrane of rat cortical collecting tubule. *Am J Physiol* 259: F494–F502, 1990
132. Frindt G, Houde V, Palmer LG: Conservation of Na+ vs. K+ by the rat cortical collecting duct. *Am J Physiol Renal Physiol* 301: F14–F20, 2011
133. Kotchen TA, Guthrie GP Jr, Galla JH, Luke RG, Welch WJ: Effects of NaCl on renin and aldosterone responses to potassium depletion. *Am J Physiol* 244: E164–E169, 1983



Osmotic Homeostasis

John Danziger and Mark L. Zeidel

Abstract

Alterations in water homeostasis can disturb cell size and function. Although most cells can internally regulate cell volume in response to osmolar stress, neurons are particularly at risk given a combination of complex cell function and space restriction within the calvarium. Thus, regulating water balance is fundamental to survival. Through specialized neuronal “osmoreceptors” that sense changes in plasma osmolality, vasopressin release and thirst are titrated in order to achieve water balance. Fine-tuning of water absorption occurs along the collecting duct, and depends on unique structural modifications of renal tubular epithelium that confer a wide range of water permeability. In this article, we review the mechanisms that ensure water homeostasis as well as the fundamentals of disorders of water balance.

Clin J Am Soc Nephrol 10: 852–862, 2015. doi: 10.2215/CJN.10741013

Crawling out on dry land some millions of years later, terrestrial forms were faced with the diametrically opposite problems, at least with respect to water. Fluid conservation, rather than fluid elimination, was the major concern. Instead of discarding their now unnecessary pressure filters and redesigning their kidneys as efficient secretory organs, the terrestrial vertebrates modified and amplified their existing systems to salvage the precious water of the filtrate.

—Robert F. Pitts (1)

So wrote the great physiologist Robert F. Pitts describing the evolution of organisms from the ocean to land (1). Marine animals survive in the high tonicity of seawater (500–1000 mOsm/kg) through a variety of mechanisms. The shark maintains a high tonicity in its body fluids (2,3), whereas dolphins absorb water from foodstuffs while producing a highly concentrated urine through complex multilobed reniculate kidneys (4). For those of us on land, however, the challenge is not only water conservation but also water elimination, in our world of coffee shops, bottled water, and “hydration for health” philosophies.

Water is the most abundant component of the human body, constituting approximately 50%–60% of body weight. Cell membranes, which define the intracellular compartment, and the vascular endothelium, which defines the intravascular component, are both water permeable. Because the intracellular space constitutes the largest body compartment, holding approximately two thirds of body fluid, changes in water homeostasis predominantly affect cells; water excess leads to cellular swelling, and water deficit leads to cellular shrinkage. For every 1 liter of water change, approximately 666 ml affect the cellular space, with only about 110 ml affecting the vascular space.

Although cells have an innate capacity to respond to changes in cell volume when extracellular osmolality changes, the body protects cells primarily by tightly

regulating extracellular osmolality. The amount of body water remains remarkably stable despite a huge range of water intake and a multitude of routes for water loss, including the respiratory and gastrointestinal tract, skin, and the kidneys. In this review, we explore the mechanisms that allow our bodies to respond to a wide range of external influences, fine-tuning the exact amount of urinary water excretion to match the body's immediate needs.

Maintaining Brain Cell Size

With a plethora of capillaries descending through the subarachnoid space into the parenchyma, the brain is remarkably vascular. Astrocytes, star-shaped neuronal cells, encapsulate the capillaries, forming a “blood-brain barrier” and controlling many important neurologic functions. Although previously thought to be impermeable (5,6), the discovery of aquaporin (AQP) channels within the astrocyte has elucidated the water permeability of this barrier (7) (Figure 1). AQP4 localizes to the perivascular and subpial aspects of astrocytes, and controls both water efflux and influx, as well as regulates potassium homeostasis, neuronal excitability, inflammation, and neuronal signaling (8). By controlling water movement from brain parenchyma into the systemic circulation, AQP4 regulates brain water content and volume (9). By controlling water influx, AQP4 plays a role in the signaling cascade that occurs in the setting of hypo-osmolar-induced cerebral edema (10).

Because the amount of intracellular water affects the concentration of intracellular contents and cell size, changes in osmolality can disturb the complex signaling network that orchestrates cell function. Given the complexity of brain function, even minor changes in neuron ionic composition and size can have profound effects on the processing and transmission of neuronal signals. Consequently, the brain has developed complex osmoregulatory mechanisms to defend against changes in plasma osmolality. Within minutes

Department of
Medicine, Beth Israel
Deaconess Medical
Center, Boston,
Massachusetts

Correspondence:

Dr. John Danziger,
Department of
Medicine, Beth Israel
Deaconess Medical
Center, 185 Pilgrim
Road, Farr 8, Boston,
MA 02215. Email:
jdanzige@bidmc.
harvard.edu

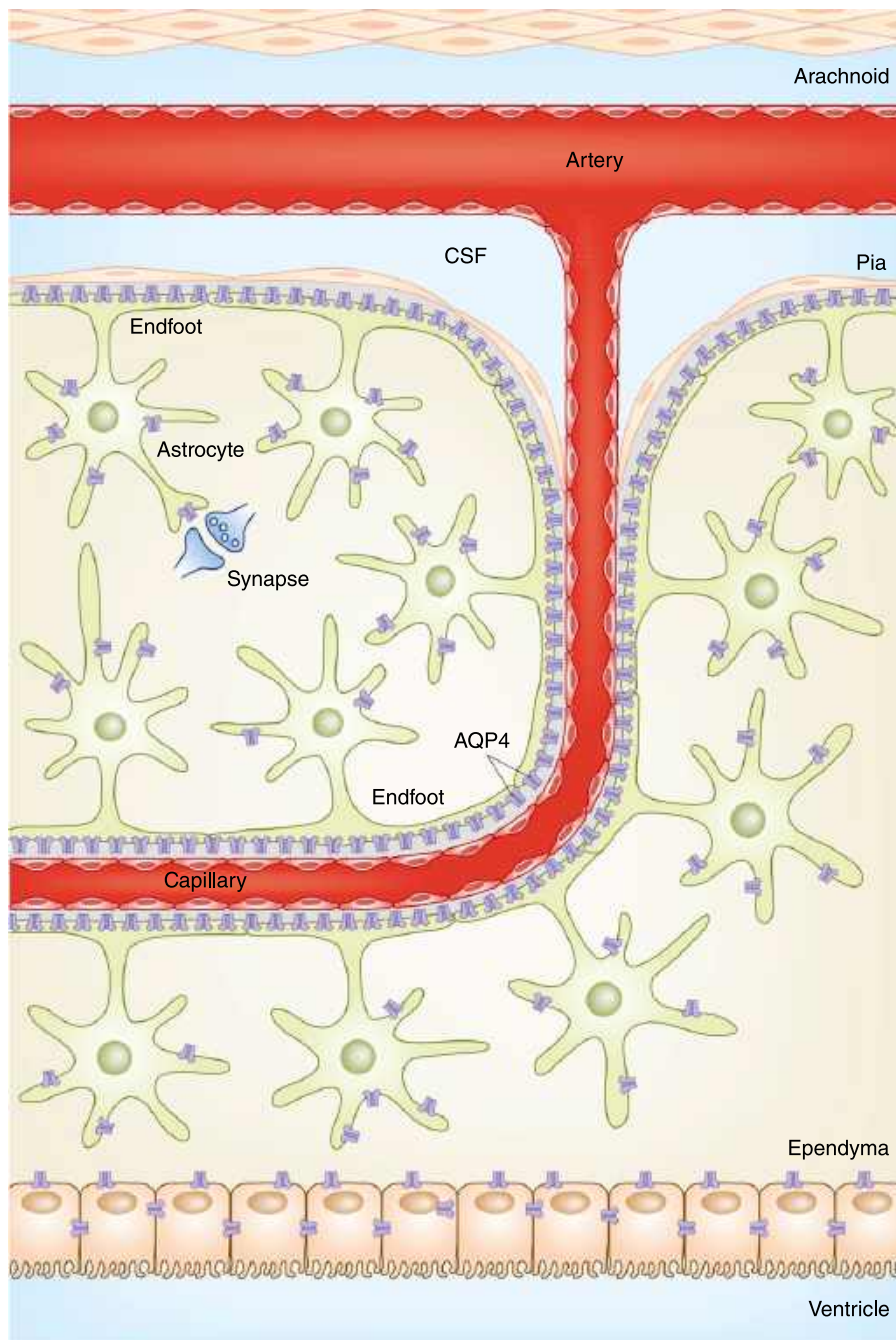


Figure 1. | The blood-brain barrier. Penetrating capillaries descend through the subarachnoid space into the parenchyma, and are encased by astrocytes, which in addition to controlling important neurologic functions, form the blood-brain barrier. AQP4 water channels along the perivascular and subpial endfoot membranes confer water permeability to the blood-brain barrier. AQP, aquaporin; CSF, cerebrospinal fluid.

of osmolar challenges, brain cells respond by either loss or accumulation of inorganic osmolytes, returning the cell size toward normal (11). In the setting of hypotonicity, as shown in Figure 2, the rapid swelling of the cell activates quiescent cell membrane channels and leads to immediate Cl^- , K^+ , and attendant water loss, a process termed *regulatory volume decrease*. Over the subsequent 24 hours, the cells lose further organic solutes, such as myo-inositol, and amino acids, such as glutamine, glutamate, and taurine. With hyperosmolar-induced cell shrinkage, brain cells

respond with immediate uptake of surrounding Na^+ , K^+ , and Cl^- , correcting cell volume in a process termed *regulatory volume increase* (12). With more prolonged exposure, organic solute concentrations within the cells rise, replacing the high levels of ions.

Despite these important cell protective mechanisms, alterations in plasma osmolality can have disastrous consequences. The classic neurologic symptoms of hypo-osmolality, including headache, nausea, vomiting, and if severe enough, seizures, are generally thought to occur at a serum sodium

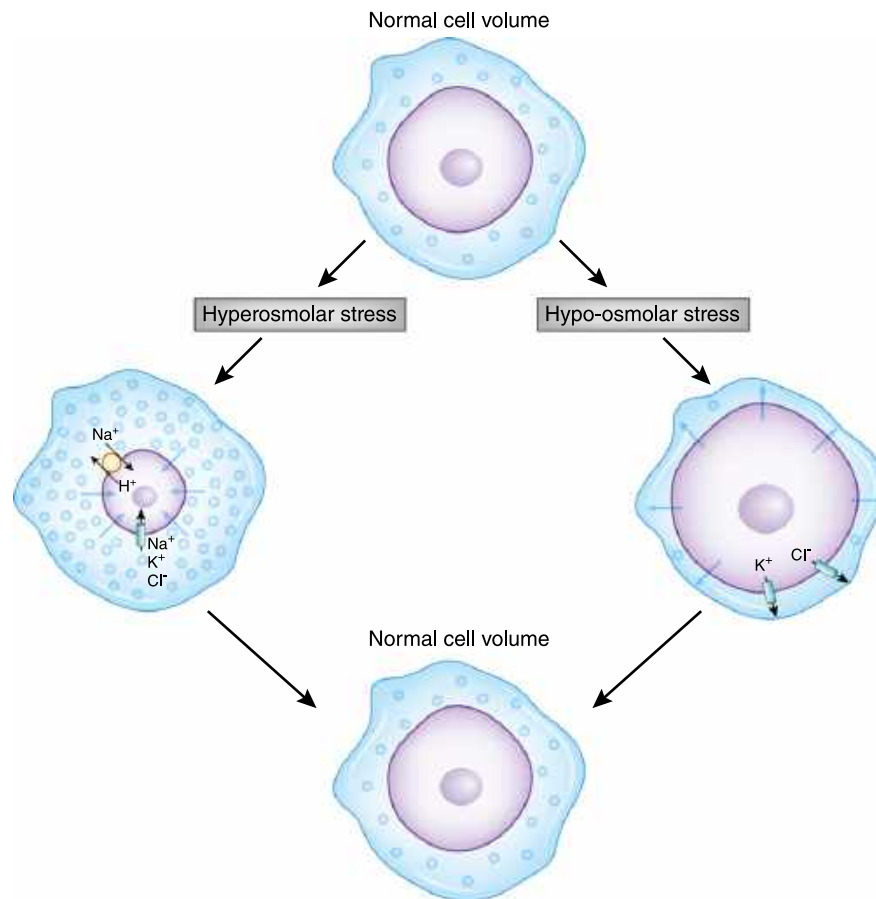


Figure 2. | Cells regulate their internal volume in response to osmotic stress by activation of membrane carrier proteins and channels. In this figure, a normal cell is challenged by either a hyperosmolar (left) or hypo-osmolar (right) milieu. In the setting of hyperosmolar stress, whereby the cell shrinks with water egress, neurons then respond by rapidly accumulating Na^+ , K^+ , and Cl^- ions, followed by the production of intracellular organic solutes. The increase of intracellular solute content then draws water in to normalize the concentrations across the cell membrane, thereby restoring cell size. In the setting of hypo-osmolar-induced swelling, activation of K^+ and Cl^- channels, as well as the K^+ - Cl^- cotransporter, lead to solute and consequent water loss, thereby restoring cell volume.

of 125 mEq/L, although with a wide range of sensitivities that are greatly affected by the rate of osmolality change. More mild changes of plasma osmolality are also associated with neurologic symptoms, including gait instability, memory impairment, and cognitive decline. Certain groups have an increased sensitivity to changes in plasma osmolality. Children are considered at increased risk of hypo-osmolar encephalopathy, possibly because of the relatively larger brain to intracranial volume compared with adults (13). Conversely, because the brain begins to atrophy in the sixth decade, elderly individuals may be at a lower risk of severe complications from acute hyponatremia. In addition to age, sex is also considered an important determinant of neurologic sensitivity. The vast majority of reported cases of postoperative hyponatremia resulting in fatal outcomes have been in women (14), including postpartum and postmenopausal women (15).

Unlike the brain swelling associated with hypo-osmolality, the brain shrinks in hypertonic conditions. The protective reflex of intense thirst may disappear as hypertonicity worsens, replaced by somnolence, confusion, and muscle weakness (16). If severe enough, the shrinking brain will pull away from the calvarium, tearing the rich capillary plexus, and causing subarachnoid hemorrhage, cerebral bleeding,

and death. The highest reported serum sodium in the adult literature remains 255 mEq/L, a consequence of drinking salty water as part of a fatal exorcism ritual (17). Presumably due to the use of table salt as a common antiemetic, fatal salt ingestion, either accidentally or voluntarily, is well reported (18), as is accidental iatrogenic administration (19). Seawater drowning has also been associated with profound hypernatremia (20). In summary, despite internal cellular mechanisms to protect cell volume, cells remain at risk with alterations of water balance; consequently, preventing significant changes in plasma osmolality is critical for survival.

Sensing Changes in Body Concentration: The Osmoreceptor

The ability to internally sense plasma osmolality is fundamental to the process of water homeostasis. Much progress in explaining the mechanisms of the “osmoreceptor” has been made, as reviewed by Sharif-Naeini *et al.* (21). Specialized neurons located in several brain areas, including the organum vasculosum laminae terminalis (OVLT) (22,23) and the supraoptic (24,25) and paraventricular nuclei of the hypothalamus, are able to sense changes

in plasma osmolality, responding with complex neuronal commands. Electrophysiologic recordings from supraoptic nuclei of the hypothalamus in rats show an increasing rate of cellular depolarization in response to water deprivation (26), and a decreasing rate with water administration (27). More recent studies have shown that hyperosmolality causes osmoreceptor membrane depolarization *via* activation of nonselective calcium-permeable cation channels. It remains somewhat unresolved whether the exact stimulus is change of specific intracellular solutes associated with cell dehydration or a mechanical effect linked to cell membrane shrinkage. Identification of the transient receptor potential vanilloid (TRPV) family of cation channels as a potential “mechanic-stretch” receptor (28) has added support to the concept of osmosensing as a mechanical process (Figure 3), and polymorphisms have been linked to hyponatremia (29). Shrinking of OVLT neurons, either by dehydration or by negative suction pressure, stimulates cell activation *via* TRPV1 (30). The importance of cell volume in neuronal activation would explain why ineffective osmoles that cross the cell membrane, such as urea and glucose (in the presence of insulin), do not activate the osmoreceptor. The osmoreceptor, likely because of its role in orchestrating the pathways of water retention, has a blunted regulatory volume decrease response, whereby its own shrinkage due to hyperosmolality is maintained, allowing sustained stimulation of thirst and vasopressin release until the plasma osmolality can be corrected (30). In the following sections, we discuss how the osmoreceptor regulates thirst and vasopressin (synonymously known as antidiuretic hormone) release.

Thirst

The sensation of thirst is the experiential component of the complex physiologic drive to drink. Neuroimaging studies have localized the anatomic origin of thirst, with

hyperosmolality stimulating activity in the anterior wall of the third ventricle, the anterior cingulate, parahippocampal gyrus, insula, and the cerebellum (31). These brain regions are also associated with complex functions, including emotional behavior and thought, perhaps explaining why the perception of thirst, in addition to its physiologic basis, is so connected to social and behavioral mores.

Hypertonicity is a reproducible stimulus of thirst. The osmolar threshold for thirst has traditionally been considered to be approximately 5 mOsm/kg above the threshold for vasopressin release, although some suggest similar set points (32). A higher thirst threshold allows vasopressin titration of urinary water excretion without the need to be constantly drinking. Responding to increasing osmolality, OVLT osmoreceptors relay stimuli to the insula and cingulate cortices *via* several medially-located thalamic nuclei, stimulating thirst (33). Upon drinking, the sensation of thirst is quenched almost immediately, suggesting that a direct satiating effect of water on the tongue and buccal membrane as well as cognitive awareness of fluid intake might explain the resolution of thirst. In addition, the recent recognition of peripheral osmoreceptors located within the gastrointestinal tract and portal venous system suggest a local mechanism that directly senses gastric water absorption (34). TRPV-positive neurons within the thoracic ganglia innervating the liver detect changes in local osmolality, and can stimulate a wide array of physiologic responses, including modulation of BP (35), metabolism (36), and water homeostasis. Whether these peripheral osmoreceptors might contribute to the disorders of osmolality frequently seen in patients with cirrhosis remains unknown.

In addition to osmotic stimuli, there are important non-osmotic stimuli of thirst. The hemodynamics of hemorrhage are potentially dipsogenic. Thirst on the battlefield is legendary, with exsanguinating soldiers asking for water. In animal models, hemorrhage stimulates intense water drinking (37),

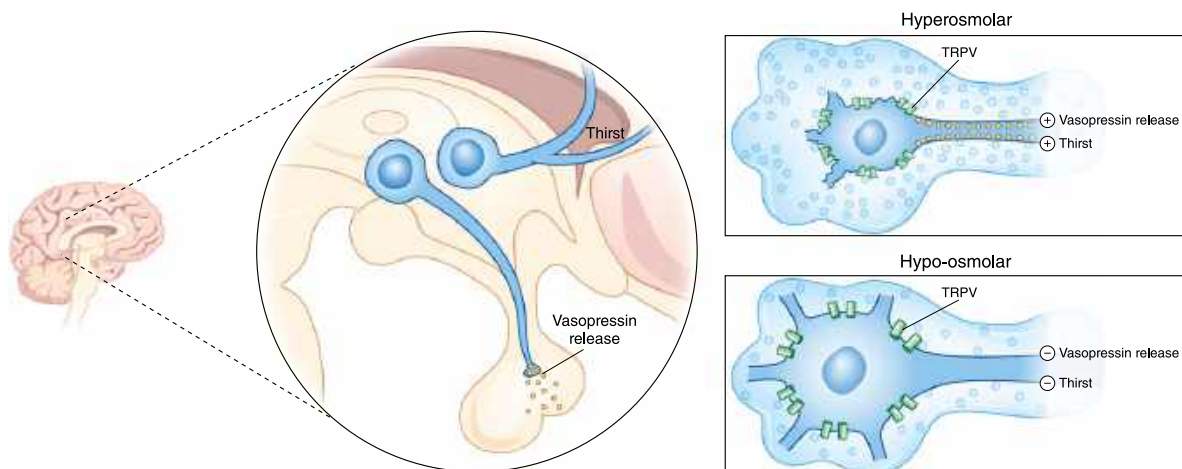


Figure 3. | Osmoreceptor functions of the OVLT nuclei and SON control thirst and vasopressin release, respectively. In response to hyperosmolar-induced cell shrinkage, specialized mechanical-stretch TRPV cation channels are activated, allowing the influx of positive charges and consequent cell depolarization, provoking action potentials that stimulate thirst and vasopressin release. Conversely, hypo-osmolar cell swelling deactivates these channels, leading to cell hyperpolarization, extinguishing thirst and vasopressin release. Although the exact role of the TRPV channel remains under investigation, its presence is critical in this mechanism. OVLT, organum vasculosum laminae terminalis; SON, supraoptic nuclei; TRPV, transient receptor potential vanilloid.

which is more easily extinguished by drinking saltwater than plain water (38,39). Angiotensin II, when injected into sensitive areas of the brain (40,41) or when injected systemically, is a powerful stimulus for water intake, as is activation of the renin-angiotensin axis (42), providing a mechanistic explanation for the association of thirst with abnormalities of body fluid volume. Thirst is a common complaint for patients with congestive heart failure (43,44), frequently plagues dialysis patients, and likely contributes to the prevalence of hyponatremia in these populations. Pharmacologic blockade of the renin-angiotensin axis, although theoretically attractive, does not seem to reduce thirst (45). In addition to disorders of fluid volume, thirst is also frequently encountered in patients with psychiatric disorders, reported in up to 25% of hospitalized patients with schizophrenia. Although this might be in part due to compulsive behavior or the anticholinergic side effects of psychotropic medications, studies have suggested an alteration of the sensation of thirst in patients with mental illness, with a lower osmolar threshold (46).

Vasopressin

Vasopressin is a potent endogenous peptide influencing a wide array of biologic functions, including regulation of water balance, BP, platelet function, and thermoregulation (47–49). It is synthesized as a prohormone in the magnocellular cell bodies of the paraventricular and supraoptic nuclei of the posterior hypothalamus, and by binding to the carrier protein neurohypophysin, it is transported along the supraoptic hypophyseal tract to the axonal terminals of magnocellular neurons in the posterior pituitary. Synthesis and storage take approximately 2 hours, with a $t_{1/2}$ of 20–30 minutes, metabolized by vasopressinases in the liver and kidney. Vasopressin acts on V_1 , V_2 , V_3 , and oxytocin-type receptors. V_1 receptors are located on the vasculature, myometrium, and platelets. V_3 receptors are mainly found in the pituitary. V_2 receptors are located along the distal tubule and collecting duct.

The most sensitive stimulus for vasopressin release is increasing plasma osmolality. Whereas normal vasopressin concentrations are 0.5–5 pg/ml in fasted, hydrated individuals (50), subtle increases in plasma osmolality, often in the range of <2% of body water, stimulate the osmoreceptor to release vasopressin, and serum concentrations rapidly increase 3-fold. The presence of stored vasopressin in the pituitary guarantees a rapid and effective mechanism of water regulation. As water is retained and the plasma osmolality returns to normal, the stimuli for vasopressin release is extinguished.

In addition, there are nonosmotic stimuli, including NE, dopamine, pain, hypoxia, and acidosis (51), and most importantly, circulatory hemodynamics. Cardiovascular collapse is associated with profound vasopressin release, with concentrations 100-fold greater than normal (52), presumably because greater vasopressin concentrations are needed to increase systolic BP than to regulate antidiuresis. Such high concentrations rapidly exhaust the pituitary vasopressin stores, and given the time-consuming nature of vasopressin production, vasopressin depletion is thought contributory to shock physiology (53). Subtle changes in body fluid volume modify the responsiveness of vasopressin release to

osmolality. Early physiologic experiments on dogs using either hemorrhage or transfusion illustrated that circulatory blood volume modified the association between plasma osmolality and vasopressin (54). For any given plasma osmolality, hemorrhage was associated with a higher vasopressin concentration, whereas transfusion was associated with a lower vasopressin concentration. In these experiments, hemorrhage and transfusion were associated with a change in left atrial pressure, but not BP.

Although myriad terms, such as *intravascular volume*, *effective arterial volume*, or *circulatory volume*, have been used to describe the component of body fluid that effectively perfuses critical organs, these terms imply that the vascular compartment is readily measurable, a feat that is difficult in the laboratory and impossible at the bedside. Furthermore, because the vascular endothelium is freely permeable to water and sodium, the intravascular and interstitial compartments freely and dynamically communicate, further limiting the idea of a separate, quantifiable intravascular space. Instead, because pressure receptors located in the heart and carotid arteries and flow receptors in the juxtaglomerular apparatus are the sensors for body fluid volume, we favor the simple term *sensed volume* (55). Arterial baroreceptors, through cranial nerves IX and X, communicate with the hypothalamus and can modify vasopressin release. Sensed volume depletion, in the setting of true volume depletion (*e.g.*, diarrhea or vomiting) or volume overload (*e.g.*, heart failure and cirrhosis), both amplify the sensitivity to vasopressin so that for any given plasma osmolality, the urinary osmolality is greater.

In summary, the osmoreceptor is stimulated by both osmotic and nonosmotic stimuli to initiate thirst and to release vasopressin in order to maintain water balance.

A Highly Concentrated Medulla

We previously described how the body senses and responds to changes in plasma osmolality. Next we turn to the final steps of osmotic homeostasis: renal water retention or excretion. Having a highly concentrated medullary interstitium is essential for water conservation, providing the osmotic force for water egress from filtered renal tubular fluid. The medulla, reaching up to four times the concentration of the surrounding interstitial fluid, is like a concentration oasis or a pocket of hypertonic fluid within a deeply vascular organ unprotected by a barrier epithelium. The generation and maintenance of the medullary interstitial gradient is one of the fundamental teachings of renal physiology (Figure 4).

Generating the medullary concentration depends on three important structural modifications of the renal tubule. First, a hairpin loop in the renal tubule allows solute and water exchange between the descending thin limb and the ascending thick limb. Second, the combination of the highly energy-dependent Na/K-ATPase and the NaK2Cl cotransporter, along with the apical water impermeability of the thick ascending limb, drive solute without water egress from the medulla. Third, because the descending limb is water permeable, the exiting sodium from the thick ascending limb creates a concentration gradient that pulls water from the descending limb, and as that tubular fluid then moves into the ascending limb, the NaK2Cl cotransporter is presented with increasingly concentrated tubular fluid, further generating

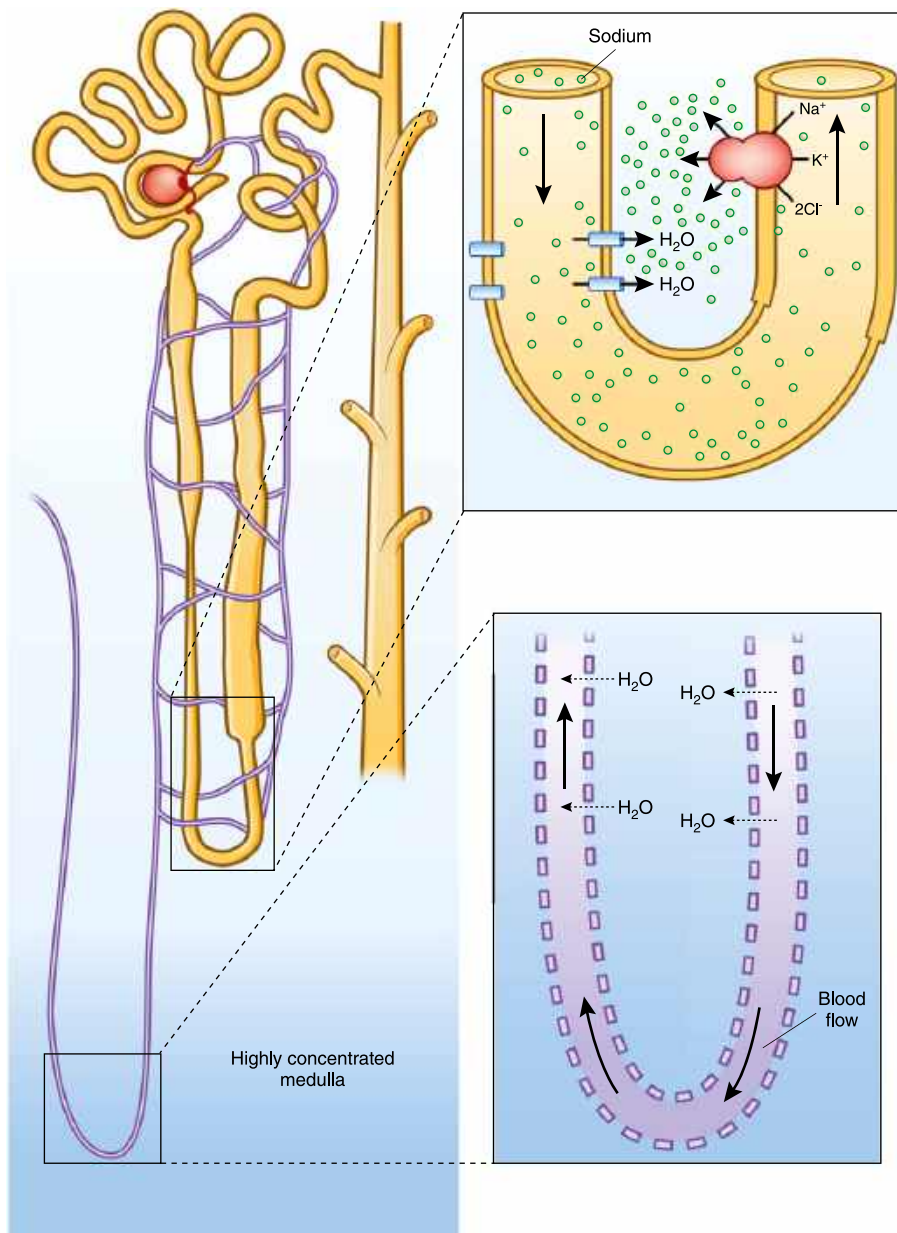


Figure 4. | The medullary interstitium has a concentration >4 times that of its surrounding fluid, and must be both generated and maintained. The countercurrent multiplier, composed of a hairpin tubule loop with a water-permeable descending limb juxtaposed against an impermeable ascending limb with a highly active Na-K-2Cl pump, generates the concentration gradient. A separate hairpin loop within the tubular capillary system allows shunting of water from the descending limb to the ascending limb preventing the dilution of the medullary gradient. This process, countercurrent exchange, maintains the medullary concentration.

more of an interstitial concentration. This process, termed *countercurrent multiplication*, is responsible for generation of approximately one half (600 mOsm/kg) of the maximal medullary concentration gradient (1200 mOsm/kg), with the remainder being generated by urea recycling (56).

Given that the kidneys receive approximately 25% of cardiac output, with the potential to rapidly wash away any area of hyperosmolarity, maintaining the medullary concentration is fundamental. There are two major mechanisms to prevent medullary washout. First, the majority of renal blood flow is directed to superficial glomeruli limited to the outer cortex, with <2% perfusing the deep

medullary glomeruli (57,58). Second, for the vasa rectae that descend into the medulla, a hairpin loop prevents medullary dilution, a process known as *countercurrent exchange*. In a manner similar to the vascular structure of a penguin's webbed foot that allows heat conservation despite walking on ice, whereby the warmth of descending blood shuttles to the ascending limb and bypasses the colder distal loop, the vasa recta's hairpin loop prevents water from reaching the distal aspects of the circuit, preventing medullary washout (59). In essence, these mechanisms shunt water away from the highly concentrated deep medulla, protecting it as a pocket of highly concentrated fluid. This combination

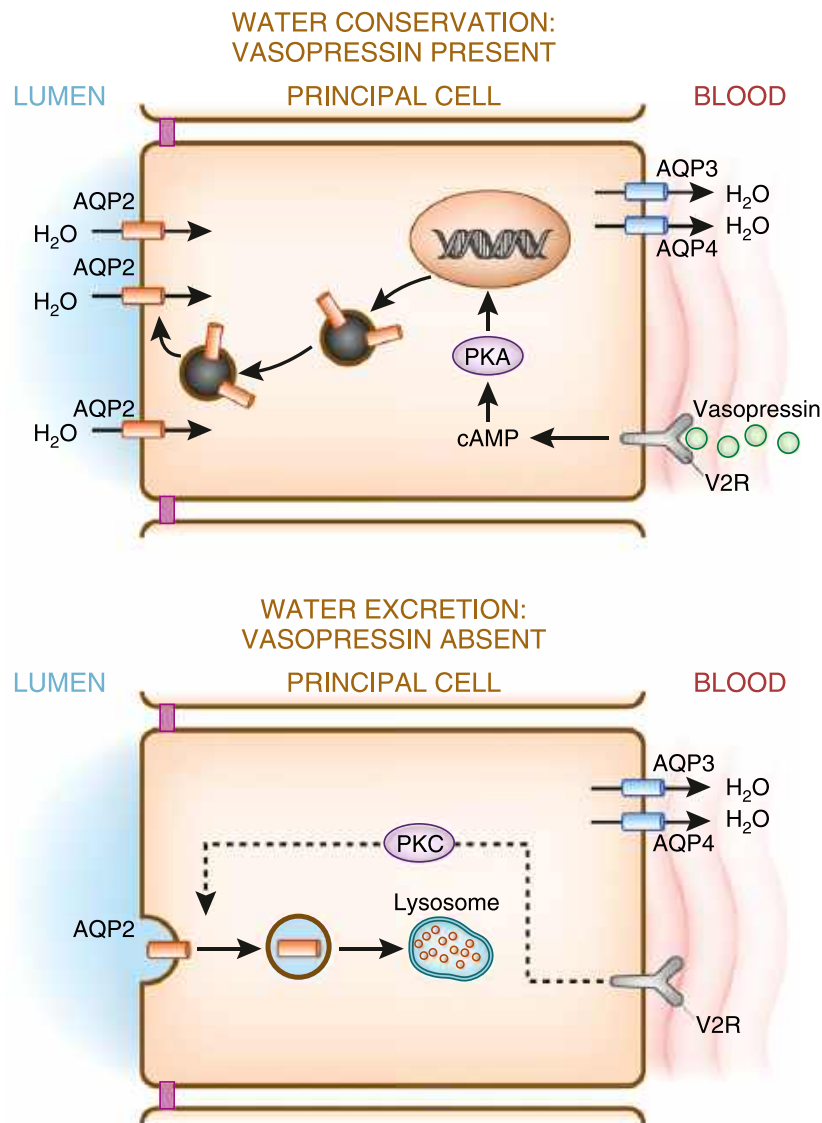


Figure 5. | Vasopressin regulates AQP2 expression. In the presence of vasopressin, increased production of cAMP activates PKA, which in turn phosphorylates stored AQP-containing vesicles, and targets them to the apical membrane, increasing its water permeability, and facilitating water reclamation from the lumen. In the absence of vasopressin, AQP2 is endocytosed and internally degraded, conferring water impermeability to the apical membrane, thereby maximizing water excretion. AQP3 and AQP4, constitutively expressed on the basolateral membrane, allow water egress from the cell. PKA, protein kinase A; V₂R, vasopressin 2 receptor.

of building and maintaining a concentrated medulla provides the force for tubular water egress and allows the fine-tuning of water balance, discussed next.

Fine-Tuning Water Balance in the Collecting Duct

The ability of the nephron to excrete a urine that is more concentrated than the plasma (water reabsorption) or more dilute than the plasma (water excretion) relies on the presence of nephron segments that are extremely permeable to water, as well as segments that are nearly impermeable. To excrete dilute urine, the collecting duct must be able to maintain an almost 30-fold concentration gradient between the dilute urinary filtrate and the surrounding highly concentrated medullary interstitium. Conversely, in order to conserve water, the collecting duct must alter its water permeability, allowing the egress of filtrate water into the

more concentrated interstitium. The water permeabilities of the different sections of the tubule are determined by the presence or absence of important structural modifications that control both the paracellular and transcellular routes of flow.

Tight junction proteins, including cytoplasmic scaffolding proteins, transmembrane proteins, and signaling proteins, act like a biologic zipper, controlling movement of water and solutes in the intercellular passageway (60). Zona occludens protein-1 functions as a scaffold protein, anchoring to other transmembrane proteins and the actin cytoskeleton, helping to seal the intercellular space. The expression of zona occludens protein-1 may respond directly to changes in medullary tonicity (61), suggesting a local level of permeability regulation. Claudins are key integral membrane proteins that function as high-conductance cation pores, regulating the transcellular movement of sodium, magnesium, and calcium

(62). In addition to their role in the impermeability of the renal tubule, the tight junctions control gastrointestinal permeability (63) and have been associated with a wide range of diarrheal illness, including Crohn's disease (64,65). The expression of tight junction proteins increases along the length of the tubule, particularly along the thick ascending limb and the collecting duct (66,67).

In addition to controlling the paracellular route, the collecting duct must prevent the transcellular movement of water. Recent work has provided a mechanistic explanation for how barrier epithelial cells achieve this transcellular impermeability (68–70). Although once thought to be simply due to the depth of the cell barrier, important modifications within the apical cell membrane are likely responsible for barrier impermeability (71,72). Barrier epithelia segregate high levels of glycosphingolipid, which entraps cholesterol, as well as long, relatively saturated fatty acid-laden triglycerides, in their outer leaflets. This composition leads to tight packing of the triglycerides, so that nearly all of the surface is composed of phosphate headgroups, which impede water flow. Water that does find the surface and penetrates has difficulty diffusing across the space between the chains because of tight packing caused by cholesterol (73,74).

Finally, water movement across the renal tubule also depends on the presence of AQP channels, as reviewed by Agre (75). AQP1 is constitutively present in the apical and

the basolateral membranes of the proximal tubule and descending limb, providing a route for transcellular movement, but is absent in the thick ascending limb. AQP2, which is expressed along the apical membrane of collecting duct principal cells, is regulated by vasopressin. Upon binding to its receptor in the basolateral membrane, vasopressin initiates a complex cascade of signals that ultimately result in the movement of AQP2 channels to the apical membrane, rendering the cell water permeable. The biologic details of this complex mechanism have largely been elucidated. As seen in Figure 5, binding of vasopressin to the vasopressin V_2 receptor on the basolateral membrane activates adenylate cyclase, increasing intracellular cAMP levels, activating protein kinase A, and leading to the translocation of AQP2 bearing vesicles to the apical membrane. Upon withdrawal of vasopressin, AQP2 is internalized into intracellular storage vesicles. In addition to the short-term regulation of AQP2 trafficking, vasopressin also influences the long-term expression of AQP2 in collecting ducts, increasing their abundance. AQP2 expression is also thought to be controlled by vasopressin-independent mechanisms, including other transcription factors (76), oxytocin (77), and possibly the novel hormone secretin (78).

As seen in Figure 6, the distribution of tight junctions and AQP channels, along with unique “barrier” qualities of renal tubular epithelium, determine the water permeability of the

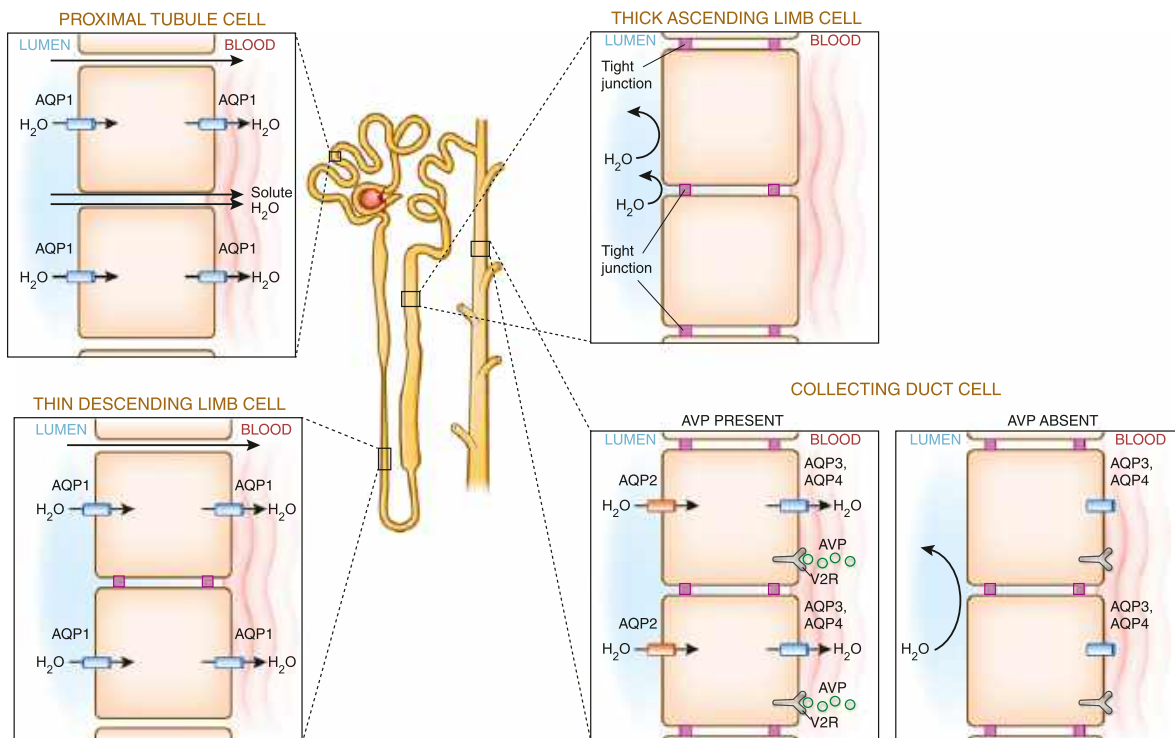


Figure 6. | Water permeability along the tubule is determined by the presence or absence of intracellular tight junctions and AQP water channels. AQP1, along the proximal tubule and thin descending limb, is constitutively expressed, whereas AQP2, in the collecting duct, is under the control of vasopressin. The presence of AQP1 and the absence of tight junctions render the proximal tubule permeable, facilitating filtered solute and water reclamation (91). In the thin descending limb, the presence of AQP1 and tight junctions (claudin 2) render it water permeable but solute impermeable (92). Conversely, the impermeability of the thick ascending limb results from extensive tight junctions and absent AQP channels. The collecting duct is unique in its homeostatic responsiveness. In times of water conservation, vasopressin (AVP) binds to vasopressin 2 receptors (V_2R), inducing AQP2 channel expression and consequent water retention, and in times of water excess, AQP2 retreats from the apical membrane due to vasopressin's absence.

renal tubule. The collecting duct is unique in its capacity to rapidly alter its water permeability under the tutelage of vasopressin, allowing fine-tuning of water excretion and guarding water homeostasis.

Clinical Correlation

Diabetes insipidus, a failure of water conservation resulting in hyperosmolality and compensatory polydipsia, is frequently encountered in clinical practice. Central diabetes insipidus can result from traumatic, surgical, or ischemic injury at any site of vasopressin production, but is most often idiopathic, possibly due to autoimmune destruction of vasopressin (79). Hereditary forms, termed *familial neurohypophyseal diabetes insipidus*, are caused by mutations in the vasopressin gene, resulting in protein misfolding and degeneration of the vasopressin-producing magnocellular neurons. Genetic abnormalities are also associated with nephrogenic diabetes insipidus (80), with mutations in the vasopressin 2 receptor gene as the most common cause. Protein misfoldings trap the vasopressin 2 receptor gene within the cell's endoplasmic reticulum, preventing it from docking with circulating vasopressin (81). These mutations are inherited in an X-linked pattern; hence, male individuals tended to have more pronounced concentration defects, whereas female individuals are usually asymptomatic. Mutations in the AQP2 gene, which can be inherited in a recessive or dominant fashion, are associated with defects in trafficking of the water channel to the apical membrane. In addition to these genetic causes, lithium use frequently causes diabetes insipidus, occurring in approximately 40% of chronic lithium users (82). It is associated with downregulation of AQP2 and cellular remodeling of the collecting duct. The route of lithium toxicity is thought to be due to cellular uptake *via* the epithelial Na channel (83), and although experimental data suggest that amiloride administration may prevent lithium nephrotoxicity (84), clinical data are lacking.

Hyponatremia is the most common electrolyte disturbance (85) and results from water intake, either orally or intravenously, in excess of excretion. For normal individuals, a water load will extinguish the osmoreceptor stimulation of thirst and vasopressin release, allowing for dilution of the urine down to <50 mOsm/kg, and rapid water excretion. Given that the average solute load of average diets is approximately 800 mOsm, primarily in the form of protein and sodium, most individuals can excrete up to 16 liters of water, and thus can drink similar amounts before becoming hyponatremic. The classic disorders of "tea and toast" or "beer potomania" occur in the setting of low-solute diets (*i.e.*, carbohydrates that are rapidly converted to water without providing solute) combined with high water intake, thus allowing hyponatremia to develop at much more modest amounts of water intake. For true psychogenic polydipsia, as defined by an ability to overwhelm the kidney's capacity to excrete water through dilute urine, patients must drink huge amounts of fluid. Hyponatremia with a urine osmolality >100 mOsm/kg signifies the presence and action of vasopressin. Because serum osmolality is the normal driver for vasopressin release, its presence at low serum osmolality suggests concentration-independent mechanisms of vasopressin release. As noted above, sensed volume depletion can stimulate vasopressin release. This can occur in

volume depletion (*e.g.*, diarrhea or vomiting) or volume overload (*e.g.*, cirrhosis or congestive heart failure). Conversely, the syndrome of inappropriate antidiuretic hormone (SIADH) secretion manifests as an inability to excrete water due to insuppressible vasopressin activity. The diagnosis of SIADH requires the absence of sensed volume depletion, and an inappropriately concentrated urine in the setting of hypo-osmolality, and occurs in a wide range of settings, including neurologic and pulmonary disease, medications, pain, and nausea (86). Recent gain-of-functions mutations in the vasopressin gene have been described, causing a "SIADH-like" clinical picture with undetectable vasopressin levels, termed *nephrogenic syndrome of inappropriate antidiuresis* (87).

Multiple studies have linked hyponatremia to increased mortality, with an increased risk ranging from 2-fold (88) to as much as 60-fold (89). Given the wide range of underlying pathologies potentially associated with hyponatremia, and the difficulty in adequately controlling for residual confounding, these observational studies should be interpreted with some caution. Although most studies have shown a linear inverse effect of decreasing sodium with mortality, recent studies have suggested a parabolic phenomenon, whereby the increased mortality associated with serum sodium in the mid-120 mEq/l range dissipates at concentrations <120 mEq/l (90). Given the risks associated with correcting hyponatremia, including central pontine myelinosis and volume overload, prospective studies are needed to further clarify the relationship of hyponatremia to outcomes.

In summary, water homeostasis depends on a functional and sensitive osmoreceptor, intact vasopressin and thirst mechanisms, and a renal tubule that can respond to the tightly orchestrated commands that dictate water retention or excretion.

Acknowledgments

J.D. is supported by a Normon S. Coplon Extramural Grant from Satellite Healthcare.

Disclosures

None.

References

1. Pitts RF: *Physiology of the Kidney and Body Fluids*, Chicago, Year Book Medical Publishers, 1963
2. Epstein FH: The shark rectal gland: A model for the active transport of chloride. *Yale J Biol Med* 52: 517–523, 1979
3. Silva P, Stoff J, Field M, Fine L, Forrest JN, Epstein FH: Mechanism of active chloride secretion by shark rectal gland: Role of Na-K-ATPase in chloride transport. *Am J Physiol* 233: F298–F306, 1977
4. Ortiz RM: Osmoregulation in marine mammals. *J Exp Biol* 204: 1831–1844, 2001
5. Crone C, Olesen SP: Electrical resistance of brain microvascular endothelium. *Brain Res* 241: 49–55, 1982
6. Crone C, Christensen O: Electrical resistance of a capillary endothelium. *J Gen Physiol* 77: 349–371, 1981
7. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP: Specialized membrane domains for water transport in glial cells: High-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci* 17: 171–180, 1997
8. Nagelhus EA, Ottersen OP: Physiological roles of aquaporin-4 in brain. *Physiol Rev* 93: 1543–1562, 2013
9. Haj-Yasein NN, Vindedal GF, Eilert-Olsen M, Gundersen GA, Skare Ø, Laake P, Klungland A, Thorén AE, Burkhardt JM, Ottersen OP, Nagelhus EA: Glial-conditional deletion of

- aquaporin-4 (Aqp4) reduces blood-brain water uptake and confers barrier function on perivascular astrocyte endfeet. *Proc Natl Acad Sci U S A* 108: 17815–17820, 2011
10. Thrane AS, Rappold PM, Fujita T, Torres A, Bekar LK, Takano T, Peng W, Wang F, Rangroo Thrane V, Enger R, Haj-Yasein NN, Skare Ø, Holen T, Klungland A, Ottersen OP, Nedergaard M, Nagelhus EA: Critical role of aquaporin-4 (AQP4) in astrocytic Ca²⁺ signaling events elicited by cerebral edema. *Proc Natl Acad Sci U S A* 108: 846–851, 2011
 11. Chamberlin ME, Strange K: Anisotonic cell volume regulation: A comparative view. *Am J Physiol* 257: C159–C173, 1989
 12. Cserr HF, DePasquale M, Nicholson C, Patlak CS, Pettigrew KD, Rice ME: Extracellular volume decreases while cell volume is maintained by ion uptake in rat brain during acute hypernatremia. *J Physiol* 442: 277–295, 1991
 13. Arieff AI, Ayus JC, Fraser CL: Hyponatremia and death or permanent brain damage in healthy children. *BMJ* 304: 1218–1222, 1992
 14. Ayus JC, Arieff AI: Chronic hyponatremic encephalopathy in postmenopausal women: Association of therapies with morbidity and mortality. *JAMA* 281: 2299–2304, 1999
 15. Ayus JC, Wheeler JM, Arieff AI: Postoperative hyponatremic encephalopathy in menstruant women. *Ann Intern Med* 117: 891–897, 1992
 16. Snyder NA, Feigl DW, Arieff AI: Hyponatremia in elderly patients. A heterogeneous, morbid, and iatrogenic entity. *Ann Intern Med* 107: 309–319, 1987
 17. Ofra Y, Lavi D, Opher D, Weiss TA, Elinav E: Fatal voluntary salt intake resulting in the highest ever documented sodium plasma level in adults (255 mmol L⁻¹): A disorder linked to female gender and psychiatric disorders. *J Intern Med* 256: 525–528, 2004
 18. Addelman M, Pollard A, Grossman RF: Survival after severe hyponatremia due to salt ingestion by an adult. *Am J Med* 78: 176–178, 1985
 19. Calvin ME, Knepper R, Robertson WO: Hazards to health. Salt poisoning. *N Engl J Med* 270: 625–626, 1964
 20. Ellis RJ: Severe hyponatremia from sea water ingestion during near-drowning in a hurricane. *West J Med* 167: 430–433, 1997
 21. Sharif-Naeini R, Ciura S, Zhang Z, Bourque CW: Contribution of TRPV channels to osmosensory transduction, thirst, and vasopressin release. *Kidney Int* 73: 811–815, 2008
 22. Vivas L, Chiaraviglio E, Carrer HF: Rat organum vasculosum laminae terminalis in vitro: Responses to changes in sodium concentration. *Brain Res* 519: 294–300, 1990
 23. Ciura S, Bourque CW: Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. *J Neurosci* 26: 9069–9075, 2006
 24. Leng G, Mason WT, Dyer RG: The supraoptic nucleus as an osmoreceptor. *Neuroendocrinology* 34: 75–82, 1982
 25. Mason WT: Supraoptic neurones of rat hypothalamus are osmosensitive. *Nature* 287: 154–157, 1980
 26. Walters JK, Hatton GI: Supraoptic neuronal activity in rats during five days of water deprivation. *Physiol Behav* 13: 661–667, 1974
 27. Brimble MJ, Dyball RE: Characterization of the responses of oxytocin- and vasopressin-secreting neurones in the supraoptic nucleus to osmotic stimulation. *J Physiol* 271: 253–271, 1977
 28. Liedtke W, Choe Y, Martí-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S: Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* 103: 525–535, 2000
 29. Tian W, Fu Y, Garcia-Elias A, Fernández-Fernández JM, Vicente R, Kramer PL, Klein RF, Hitzemann R, Orwoll ES, Wilmot B, McWeeney S, Valverde MA, Cohen DM: A loss-of-function nonsynonymous polymorphism in the osmoregulatory TRPV4 gene is associated with human hyponatremia. *Proc Natl Acad Sci U S A* 106: 14034–14039, 2009
 30. Ciura S, Liedtke W, Bourque CW: Hypertonicity sensing in organum vasculosum lamina terminalis neurons: A mechanical process involving TRPV1 but not TRPV4. *J Neurosci* 31: 14669–14676, 2011
 31. Egan G, Silk T, Zamarripa F, Williams J, Federico P, Cunningham R, Carabott L, Blair-West J, Shade R, McKinley M, Farrell M, Lancaster J, Jackson G, Fox P, Denton D: Neural correlates of the emergence of consciousness of thirst. *Proc Natl Acad Sci U S A* 100: 15241–15246, 2003
 32. Thompson CJ, Bland J, Burd J, Baylis PH: The osmotic thresholds for thirst and vasopressin release are similar in healthy man. *Clin Sci (Lond)* 71: 651–656, 1986
 33. Hollis JH, McKinley MJ, D'Souza M, Kampe J, Oldfield BJ: The trajectory of sensory pathways from the lamina terminalis to the insular and cingulate cortex: A neuroanatomical framework for the generation of thirst. *Am J Physiol Regul Integr Comp Physiol* 294: R1390–R1401, 2008
 34. Lechner SG, Markworth S, Poole K, Smith ES, Lapatsina L, Frahm S, May M, Pischke S, Suzuki M, Ibañez-Tallon I, Luft FC, Jordan J, Lewin GR: The molecular and cellular identity of peripheral osmoreceptors. *Neuron* 69: 332–344, 2011
 35. McHugh J, Keller NR, Appalsamy M, Thomas SA, Raj SR, Diedrich A, Biaggioni I, Jordan J, Robertson D: Portal osmopressor mechanism linked to transient receptor potential vanilloid 4 and blood pressure control. *Hypertension* 55: 1438–1443, 2010
 36. Boschmann M, Steiniger J, Franke G, Birkenfeld AL, Luft FC, Jordan J: Water drinking induces thermogenesis through osmosensitive mechanisms. *J Clin Endocrinol Metab* 92: 3334–3337, 2007
 37. Russell PJ, Abdelaal AE, Mogenson GJ: Graded levels of hemorrhage, thirst and angiotensin II in the rat. *Physiol Behav* 15: 117–119, 1975
 38. Stricker EM: Inhibition of thirst in rats following hypovolemia and/or caval ligation. *Physiol Behav* 6: 293–298, 1971
 39. Stricker EM: Osmoregulation and volume regulation in rats: Inhibition of hypovolemic thirst by water. *Am J Physiol* 217: 98–105, 1969
 40. el Ghissassi M, Thornton SN, Nicolaïdis S: Angiotensin II-induced thirst, but not sodium appetite, via AT1 receptors in organum cavum prelamina terminalis. *Am J Physiol* 268: R1401–R1405, 1995
 41. Weisinger RS, Blair-West JR, Burns P, Denton DA, Tarjan E: Role of brain angiotensin in thirst and sodium appetite of rats. *Peptides* 18: 977–984, 1997
 42. Leenen FH, Stricker EM: Plasma renin activity and thirst following hypovolemia or caval ligation in rats. *Am J Physiol* 226: 1238–1242, 1974
 43. Waldréus N, Sjöstrand F, Hahn RG: Thirst in the elderly with and without heart failure. *Arch Gerontol Geriatr* 53: 174–178, 2011
 44. Waldréus N, Hahn RG, Jaarsma T: Thirst in heart failure: A systematic literature review. *Eur J Heart Fail* 15: 141–149, 2013
 45. Masajtis-Zagajewska A, Nowicki M: Influence of dual blockade of the renin-angiotensin system on thirst in hemodialysis patients. *Nephron Clin Pract* 112: c242–c247, 2009
 46. Goldman MB, Luchins DJ, Robertson GL: Mechanisms of altered water metabolism in psychotic patients with polydipsia and hyponatremia. *N Engl J Med* 318: 397–403, 1988
 47. Ishikawa SE, Schrier RW: Pathophysiological roles of arginine vasopressin and aquaporin-2 in impaired water excretion. *Clin Endocrinol (Oxf)* 58: 1–17, 2003
 48. Martin PY, Abraham WT, Lieming X, Olson BR, Oren RM, Ohara M, Schrier RW: Selective V2-receptor vasopressin antagonism decreases urinary aquaporin-2 excretion in patients with chronic heart failure. *J Am Soc Nephrol* 10: 2165–2170, 1999
 49. Schrier RW: Vasopressin and aquaporin 2 in clinical disorders of water homeostasis. *Semin Nephrol* 28: 289–296, 2008
 50. Cowley AW Jr, Cushman WC, Quillen EW Jr, Skelton MM, Langford HG: Vasopressin elevation in essential hypertension and increased responsiveness to sodium intake. *Hypertension* 3: 193–199, 1981
 51. Leng G, Brown CH, Russell JA: Physiological pathways regulating the activity of magnocellular neurosecretory cells. *Prog Neurobiol* 57: 625–655, 1999
 52. Cowley AW Jr, Switzer SJ, Guinn MM: Evidence and quantification of the vasopressin arterial pressure control system in the dog. *Circ Res* 46: 58–67, 1980
 53. Sharshar T, Carlier R, Blanchard A, Feydy A, Gray F, Paillard M, Raphael JC, Gajdos P, Annane D: Depletion of neurohypophyseal content of vasopressin in septic shock. *Crit Care Med* 30: 497–500, 2002
 54. Quillen EW Jr, Cowley AW Jr: Influence of volume changes on osmolality-vasopressin relationships in conscious dogs. *Am J Physiol* 244: H73–H79, 1983

55. Danziger J, Zeidel M, Parker MJ: *Renal Physiology: A Clinical Approach*, Baltimore, Lippincott Williams and Wilkins, 2012
56. Epstein FH, Kleeman CR, Pursel S, Hendriks A: The effect of feeding protein and urea on the renal concentrating process. *J Clin Invest* 36: 635–641, 1957
57. Moffat DB, Fourman J: The vascular pattern of the rat kidney. *J Anat* 97: 543–553, 1963
58. Moffat DB, Fourman J: A vascular pattern of the rat kidney. 1963. *J Am Soc Nephrol* 12: 624–632, 2001
59. Pallone TL, Edwards A, Mattson DL: Renal medullary circulation. *Compr Physiol* 2: 97–140, 2012
60. Denker BM, Sabath E: The biology of epithelial cell tight junctions in the kidney. *J Am Soc Nephrol* 22: 622–625, 2011
61. Then C, Bergler T, Jeblick R, Jung B, Banas B, Krämer BK: Hypertonic stress promotes the upregulation and phosphorylation of zonula occludens 1. *Nephron, Physiol* 119: 11–21, 2011
62. Hou J, Rajagopal M, Yu AS: Claudins and the kidney. *Annu Rev Physiol* 75: 479–501, 2013
63. Shen L, Turner JR: Role of epithelial cells in initiation and propagation of intestinal inflammation. Eliminating the static: Tight junction dynamics exposed. *Am J Physiol Gastrointest Liver Physiol* 290: G577–G582, 2006
64. Howden CW, Gillanders I, Morris AJ, Duncan A, Danesh B, Russell RL: Intestinal permeability in patients with Crohn's disease and their first-degree relatives. *Am J Gastroenterol* 89: 1175–1176, 1994
65. Katz KD, Hollander D, Vadheim CM, McElree C, Delahunty T, Dadufalza VD, Krugliak P, Rotter JI: Intestinal permeability in patients with Crohn's disease and their healthy relatives. *Gastroenterology* 97: 927–931, 1989
66. Gonzalez-Mariscal L, Namorado MC, Martin D, Luna J, Alarcon L, Islas S, Valencia L, Muriel P, Ponce L, Reyes JL: Tight junction proteins ZO-1, ZO-2, and occludin along isolated renal tubules. *Kidney Int* 57: 2386–2402, 2000
67. Gonzalez-Mariscal L, Namorado Mdel C, Martin D, Sierra G, Reyes JL: The tight junction proteins claudin-7 and -8 display a different subcellular localization at Henle's loops and collecting ducts of rabbit kidney. *Nephrol Dial Transplant* 21: 2391–2398, 2006
68. Nagle JF, Mathai JC, Zeidel ML, Tristram-Nagle S: Theory of passive permeability through lipid bilayers. *J Gen Physiol* 131: 77–85, 2008
69. Mathai JC, Tristram-Nagle S, Nagle JF, Zeidel ML: Structural determinants of water permeability through the lipid membrane. *J Gen Physiol* 131: 69–76, 2008
70. Mathai JC, Zeidel ML: Measurement of water and solute permeability by stopped-flow fluorimetry. *Methods Mol Biol* 400: 323–332, 2007
71. Zeidel ML: Low permeabilities of apical membranes of barrier epithelia: What makes watertight membranes watertight? *Am J Physiol* 271: F243–F245, 1996
72. Rivers R, Blanchard A, Eladari D, Levieil F, Paillard M, Podevin RA, Zeidel ML: Water and solute permeabilities of medullary thick ascending limb apical and basolateral membranes. *Am J Physiol* 274: F453–F462, 1998
73. Gensure RH, Zeidel ML, Hill WG: Lipid raft components cholesterol and sphingomyelin increase H⁺/OH⁻ permeability of phosphatidylcholine membranes. *Biochem J* 398: 485–495, 2006
74. Tristram-Nagle S, Kim DJ, Akhunzada N, Kucerka N, Mathai JC, Katsaras J, Zeidel M, Nagle JF: Structure and water permeability of fully hydrated diphytanoylPC. *Chem Phys Lipids* 163: 630–637, 2010
75. Agre P: Homer W. Smith award lecture. Aquaporin water channels in kidney. *J Am Soc Nephrol* 11: 764–777, 2000
76. Hasler U, Jeon US, Kim JA, Mordasini D, Kwon HM, Féraille E, Martin PY: Tonicity-responsive enhancer binding protein is an essential regulator of aquaporin-2 expression in renal collecting duct principal cells. *J Am Soc Nephrol* 17: 1521–1531, 2006
77. Jeon US, Joo KW, Na KY, Kim YS, Lee JS, Kim J, Kim GH, Nielsen S, Knepper MA, Han JS: Oxytocin induces apical and basolateral redistribution of aquaporin-2 in rat kidney. *Nephron, Exp Nephrol* 93: e36–e45, 2003
78. Chu JY, Chung SC, Lam AK, Tam S, Chung SK, Chow BK: Phenotypes developed in secretin receptor-null mice indicated a role for secretin in regulating renal water reabsorption. *Mol Cell Biol* 27: 2499–2511, 2007
79. Pivonello R, De Bellis A, Faggiano A, Di Salle F, Petretta M, Di Somma C, Perrino S, Altucci P, Bizzarro A, Bellastella A, Lombardi G, Colao A: Central diabetes insipidus and autoimmunity: Relationship between the occurrence of antibodies to arginine vasopressin-secreting cells and clinical, immunological, and radiological features in a large cohort of patients with central diabetes insipidus of known and unknown etiology. *J Clin Endocrinol Metab* 88: 1629–1636, 2003
80. Fujiwara TM, Bichet DG: Molecular biology of hereditary diabetes insipidus. *J Am Soc Nephrol* 16: 2836–2846, 2005
81. Morello JP, Salahpour A, Laperrière A, Bernier V, Arthus MF, Loneragan M, Petäjä-Repo U, Angers S, Morin D, Bichet DG, Bouvier M: Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. *J Clin Invest* 105: 887–895, 2000
82. Stone KA: Lithium-induced nephrogenic diabetes insipidus. *J Am Board Fam Pract* 12: 43–47, 1999
83. Christensen BM, Zuber AM, Löffing J, Stehle JC, Deen PM, Rossier BC, Hummler E: α ENaC-mediated lithium absorption promotes nephrogenic diabetes insipidus. *J Am Soc Nephrol* 22: 253–261, 2011
84. Kortenoeven ML, Li Y, Shaw S, Gaeggeler HP, Rossier BC, Wetzels JF, Deen PM: Amiloride blocks lithium entry through the sodium channel thereby attenuating the resultant nephrogenic diabetes insipidus. *Kidney Int* 76: 44–53, 2009
85. Upadhyay A, Jaber BL, Madias NE: Incidence and prevalence of hyponatremia. *Am J Med* 119[Suppl 1]: S30–S35, 2006
86. Robertson GL: Regulation of arginine vasopressin in the syndrome of inappropriate antidiuresis. *Am J Med* 119[Suppl 1]: S36–S42, 2006
87. Feldman BJ, Rosenthal SM, Vargas GA, Fenwick RG, Huang EA, Matsuda-Abedini M, Lustig RH, Mathias RS, Portale AA, Miller WL, Gitelman SE: Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med* 352: 1884–1890, 2005
88. Waikar SS, Mount DB, Curhan GC: Mortality after hospitalization with mild, moderate, and severe hyponatremia. *Am J Med* 122: 857–865, 2009
89. Anderson RJ, Chung HM, Kluge R, Schrier RW: Hyponatremia: A prospective analysis of its epidemiology and the pathogenetic role of vasopressin. *Ann Intern Med* 102: 164–168, 1985
90. Chawla A, Sterns RH, Nigwekar SU, Cappuccino JD: Mortality and serum sodium: Do patients die from or with hyponatremia? *Clin J Am Soc Nephrol* 6: 960–965, 2011
91. Kiuchi-Saishin Y, Gotoh S, Furuse M, Takasuga A, Tano Y, Tsukita S: Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments. *J Am Soc Nephrol* 13: 875–886, 2002
92. Maunsbach AB, Marples D, Chin E, Ning G, Bondy C, Agre P, Nielsen S: Aquaporin-1 water channel expression in human kidney. *J Am Soc Nephrol* 8: 1–14, 1997

Correction

Danziger J, Zeidel ML. Osmotic homeostasis. *Clin J Am Soc Nephrol* 10: 852–862, 2015.

On page 860 within the above-referenced article, the following sentence was incorrect: "Recent gain-of-functions mutations in the vasopressin gene have been described, causing a 'SIADH-like' clinical picture with undetectable vasopressin levels, termed *nephrogenic syndrome of inappropriate antidiuresis*."

The sentence should have indicated that the mutations are in the V2 vasopressin receptor gene and should have read as follows: "Recent gain-of-function mutations in the V2 vasopressin receptor gene have been described, causing a 'SIADH-like' clinical picture with undetectable vasopressin levels, termed *nephrogenic syndrome of inappropriate antidiuresis*."

Published online ahead of print. Publication date available at www.cjasn.org.



Regulation of Potassium Homeostasis

Biff F. Palmer

Abstract

Potassium is the most abundant cation in the intracellular fluid, and maintaining the proper distribution of potassium across the cell membrane is critical for normal cell function. Long-term maintenance of potassium homeostasis is achieved by alterations in renal excretion of potassium in response to variations in intake. Understanding the mechanism and regulatory influences governing the internal distribution and renal clearance of potassium under normal circumstances can provide a framework for approaching disorders of potassium commonly encountered in clinical practice. This paper reviews key aspects of the normal regulation of potassium metabolism and is designed to serve as a readily accessible review for the well informed clinician as well as a resource for teaching trainees and medical students.

Clin J Am Soc Nephrol 10: 1050–1060, 2015. doi: 10.2215/CJN.08580813

Department of
Internal Medicine,
University of Texas
Southwestern Medical
Center, Dallas, Texas

Introduction

Potassium plays a key role in maintaining cell function. Almost all cells possess an $\text{Na}^+\text{-K}^+\text{-ATPase}$, which pumps Na^+ out of the cell and K^+ into the cell and leads to a K^+ gradient across the cell membrane ($\text{K}^+_{\text{in}} > \text{K}^+_{\text{out}}$) that is partially responsible for maintaining the potential difference across the membrane. This potential difference is critical to the function of cells, particularly in excitable tissues, such as nerve and muscle. The body has developed numerous mechanisms for defense of serum K^+ . These mechanisms serve to maintain a proper distribution of K^+ within the body as well as regulate the total body K^+ content.

Internal Balance of K^+

The kidney is primarily responsible for maintaining total body K^+ content by matching K^+ intake with K^+ excretion. Adjustments in renal K^+ excretion occur over several hours; therefore, changes in extracellular K^+ concentration are initially buffered by movement of K^+ into or out of skeletal muscle. The regulation of K^+ distribution between the intracellular and extracellular space is referred to as internal K^+ balance. The most important factors regulating this movement under normal conditions are insulin and catecholamines (1).

After a meal, the postprandial release of insulin functions to not only regulate the serum glucose concentration but also shift dietary K^+ into cells until the kidney excretes the K^+ load re-establishing K^+ homeostasis. These effects are mediated through insulin binding to cell surface receptors, which stimulates glucose uptake in insulin-responsive tissues through the insertion of the glucose transporter protein GLUT4 (2,3). An increase in the activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ mediates K^+ uptake (Figure 1). In patients with the metabolic syndrome or CKD, insulin-mediated glucose uptake is impaired, but cellular K^+ uptake remains normal (4,5), demonstrating differential regulation of insulin-mediated glucose and K^+ uptake.

Catecholamines regulate internal K^+ distribution, with α -adrenergic receptors impairing and β -adrenergic receptors promoting cellular entry of K^+ . β_2 -Receptor-induced stimulation of K^+ uptake is mediated by activation of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump. These effects play a role in regulating the cellular release of K^+ during exercise (6).

Under normal circumstances, exercise is associated with movement of intracellular K^+ into the interstitial space in skeletal muscle. Increases in interstitial K^+ can be as high as 10–12 mM with severe exercise. Accumulation of K^+ is a factor limiting the excitability and contractile force of muscle accounting for the development of fatigue (7,8). Additionally, increases in interstitial K^+ play a role in eliciting rapid vasodilation, allowing for blood flow to increase in exercising muscle (9). During exercise, release of catecholamines through β_2 stimulation limits the rise in extracellular K^+ concentration that otherwise occurs as a result of normal K^+ release by contracting muscle. Although the mechanism is likely to be multifactorial, total body K^+ depletion may blunt the accumulation of K^+ into the interstitial space, limiting blood flow to skeletal muscle and accounting for the association of hypokalemia with rhabdomyolysis.

Changes in plasma tonicity and acid-base disorders also influence internal K^+ balance. Hyperglycemia leads to water movement from the intracellular to extracellular compartment. This water movement favors K^+ efflux from the cell through the process of solvent drag. In addition, cell shrinkage causes intracellular K^+ concentration to increase, creating a more favorable concentration gradient for K^+ efflux. Mineral acidosis, but not organic acidosis, can be a cause of cell shift in K^+ . As recently reviewed, the general effect of acidemia to cause K^+ loss from cells is not because of a direct $\text{K}^+\text{-H}^+$ exchange, but, rather, is because of an apparent coupling resulting from effects of acidosis on transporters that normally regulate cell pH in skeletal muscle (10) (Figure 2).

Correspondence:

Dr. Biff F. Palmer,
Department of
Internal Medicine,
University of Texas
Southwestern Medical
Center, 5323 Harry
Hines Boulevard,
Dallas, TX 75390.
Email: biff.palmer@
utsouthwestern.edu

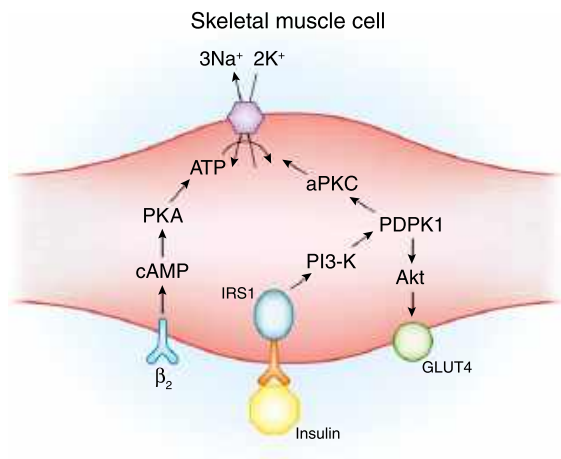


Figure 1. | The cell model illustrates β_2 -adrenergic and insulin-mediated regulatory pathways for K^+ uptake. β_2 -Adrenergic and insulin both lead to K^+ uptake by stimulating the activity of the Na^+ - K^+ -ATPase pump primarily in skeletal muscle, but they do so through different signaling pathways. β_2 -Adrenergic stimulation leads to increased pump activity through a cAMP- and protein kinase A (PKA)-dependent pathway. Insulin binding to its receptor leads to phosphorylation of the insulin receptor substrate protein (IRS-1), which, in turn, binds to phosphatidylinositol 3-kinase (PI3-K). The IRS-1-PI3-K interaction leads to activation of 3-phosphoinositide-dependent protein kinase-1 (PDK1). The stimulatory effect of insulin on glucose uptake and K^+ uptake diverge at this point. An Akt-dependent pathway is responsible for membrane insertion of the glucose transporter GLUT4, whereas activation of atypical protein kinase C (aPKC) leads to membrane insertion of the Na^+ - K^+ -ATPase pump (reviewed in ref. 3).

Intracellular K^+ serves as a reservoir to limit the fall in extracellular K^+ concentrations occurring under pathologic conditions where there is loss of K^+ from the body. The efficiency of this effect was shown by military recruits undergoing training in the summer (11). These subjects were able to maintain a near-normal serum K^+ concentration despite daily sweat K^+ losses of >40 mmol and an 11-day cumulative total body K^+ deficit of approximately 400 mmol.

Studies in rats using a K^+ clamp technique afforded insight into the role of skeletal muscle in regulating extracellular K^+ concentration (12). With this technique, insulin is administered at a constant rate, and K^+ is simultaneously infused at a rate designed to prevent any drop in plasma K^+ concentration. The amount of K^+ administered is presumed to be equal to the amount of K^+ entering the intracellular space of skeletal muscle.

In rats deprived of K^+ for 10 days, the plasma K^+ concentration decreased from 4.2 to 2.9 mmol/L. Insulin-mediated K^+ disappearance declined by more than 90% compared with control values. This decrease in K^+ uptake was accompanied by a $>50\%$ reduction in both the activity and expression of muscle Na^+ - K^+ -ATPase, suggesting that decreased pump activity might account for the decrease in insulin effect. This decrease in muscle K^+ uptake, under conditions of K^+ depletion, may limit excessive falls in extracellular K^+ concentration that occur under conditions of insulin stimulation. Concurrently, reductions in pump

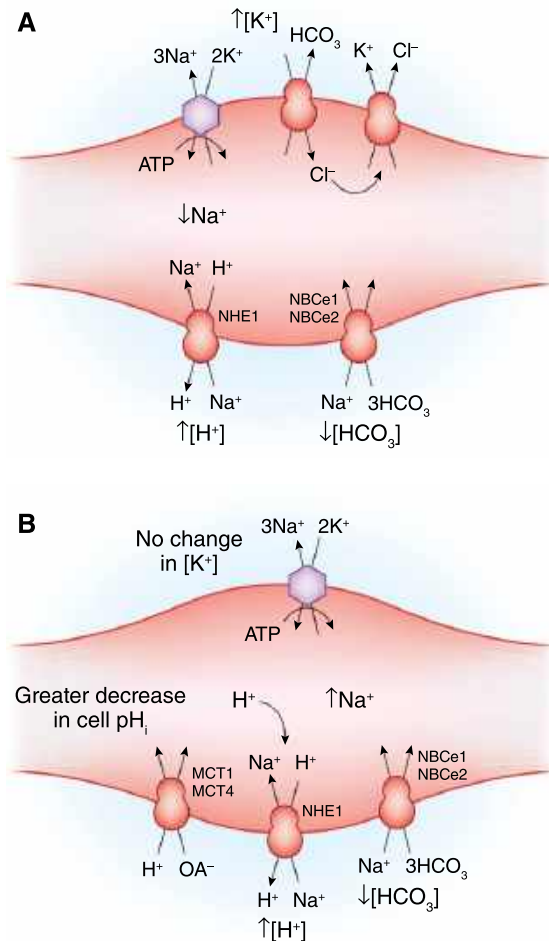


Figure 2. | The effect of metabolic acidosis on internal K^+ balance in skeletal muscle. (A) In metabolic acidosis caused by inorganic anions (mineral acidosis), the decrease in extracellular pH will decrease the rate of Na^+ - H^+ exchange (NHE1) and inhibit the inward rate of Na^+ - $3HCO_3^-$ cotransport (NBCe1 and NBCe2). The resultant fall in intracellular Na^+ will reduce Na^+ - K^+ -ATPase activity, causing a net loss of cellular K^+ . In addition, the fall in extracellular HCO_3^- concentration will increase inward movement of Cl^- by Cl^- - HCO_3^- exchange, further enhancing K^+ efflux by K^+ - Cl^- cotransport. (B) Loss of K^+ from the cell is much smaller in magnitude in metabolic acidosis caused by an organic anion. In this setting, there is a strong inward flux of the organic anion and H^+ through the monocarboxylate transporter (MCT; MCT1 and MCT4). Accumulation of the acid results in a larger fall in intracellular pH, thereby stimulating inward Na^+ movement by way of Na^+ - H^+ exchange and Na^+ - $3HCO_3^-$ cotransport. Accumulation of intracellular Na^+ maintains Na^+ - K^+ -ATPase activity, thereby minimizing any change in extracellular K^+ concentration.

expression and activity facilitate the ability of skeletal muscle to buffer declines in extracellular K^+ concentrations by donating some component of its intracellular stores.

There are differences between skeletal and cardiac muscle in the response to chronic K^+ depletion. Although skeletal muscle readily relinquishes K^+ to minimize the drop in plasma K^+ concentration, cardiac tissue K^+ content remains relatively well preserved. In contrast to the decline in activity and expression of skeletal muscle Na^+ - K^+ -ATPase, cardiac Na^+ - K^+ -ATPase pool size

increases in K^+ -deficient animals. This difference explains the greater total K^+ clearance capacity after the acute administration of intravenous KCl to rats fed a K^+ -free diet for 2 weeks compared with K^+ -replete controls (13,14). Cardiac muscle accumulates a considerable amount of K^+ in the setting of an acute load. When expressed on a weight basis, the cardiac capacity for K^+ uptake is comparable with that of skeletal muscle under conditions of K^+ depletion and may actually exceed skeletal muscle under control conditions.

Renal Potassium Handling

Potassium is freely filtered by the glomerulus. The bulk of filtered K^+ is reabsorbed in the proximal tubule and loop of Henle, such that less than 10% of the filtered load reaches the distal nephron. In the proximal tubule, K^+ absorption is primarily passive and proportional to Na^+ and water (Figure 3). K^+ reabsorption in the thick ascending limb of Henle occurs through both transcellular and paracellular pathways. The transcellular component is mediated by K^+ transport on the apical membrane $Na^+-K^+-2Cl^-$ cotransporter (Figure 4). K^+ secretion begins in the early distal convoluted tubule and progressively increases along the distal nephron into the cortical collecting duct (Figure 5). Most urinary K^+ can be accounted for by electrogenic K^+ secretion mediated by principal cells in the initial collecting duct and the cortical collecting duct (Figure 6). An electroneutral K^+ and Cl^- cotransport mechanism is also present on the apical surface of the

distal nephron (15). Under conditions of K^+ depletion, reabsorption of K^+ occurs in the collecting duct. This process is mediated by upregulation in the apically located $H^+-K^+-ATPase$ on α -intercalated cells (16) (Figure 7).

Under most homeostatic conditions, K^+ delivery to the distal nephron remains small and is fairly constant. By contrast, the rate of K^+ secretion by the distal nephron varies and is regulated according to physiologic needs. The cellular determinants of K^+ secretion in the principal cell include the intracellular K^+ concentration, the luminal K^+ concentration, the potential (voltage) difference across the luminal membrane, and the permeability of the luminal membrane for K^+ . Conditions that increase cellular K^+ concentration, decrease luminal K^+ concentration, or render the lumen more electronegative will increase the rate of K^+ secretion. Conditions that increase the permeability of the luminal membrane for K^+ will increase the rate of K^+ secretion. Two principal determinants of K^+ secretion are mineralocorticoid activity and distal delivery of Na^+ and water.

Aldosterone is the major mineralocorticoid in humans and affects several of the cellular determinants discussed above, leading to stimulation of K^+ secretion. First, aldosterone increases intracellular K^+ concentration by stimulating the activity of the $Na^+-K^+-ATPase$ in the basolateral membrane. Second, aldosterone stimulates Na^+ reabsorption across the luminal membrane, which increases the electronegativity of the lumen, thereby increasing the electrical gradient favoring K^+ secretion. Lastly, aldosterone has a direct effect on the luminal membrane to increase K^+ permeability (17).

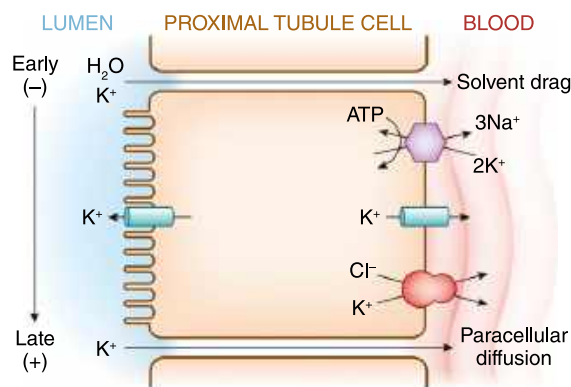


Figure 3. | A cell model for K^+ transport in the proximal tubule. K^+ reabsorption in the proximal tubule primarily occurs through the paracellular pathway. Active Na^+ reabsorption drives net fluid reabsorption across the proximal tubule, which in turn, drives K^+ reabsorption through a solvent drag mechanism. As fluid flows down the proximal tubule, the luminal voltage shifts from slightly negative to slightly positive. The shift in transepithelial voltage provides an additional driving force favoring K^+ diffusion through the low-resistance paracellular pathway. Experimental studies suggest that there may be a small component of transcellular K^+ transport; however, the significance of this pathway is not known. K^+ uptake through the $Na^+-K^+-ATPase$ pump can exit the basolateral membrane through a conductive pathway or coupled to Cl^- . An apically located K^+ channel functions to stabilize the cell negative potential, particularly in the setting of Na^+ -coupled cotransport of glucose and amino acids, which has a depolarizing effect on cell voltage.

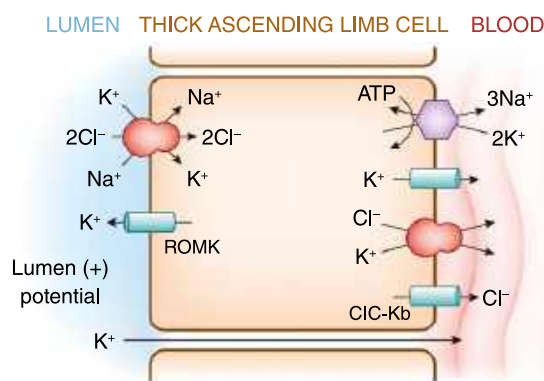


Figure 4. | A cell model for K^+ transport in the thick ascending limb of Henle. K^+ reabsorption occurs by both paracellular and transcellular mechanisms. The basolateral $Na^+-K^+-ATPase$ pump maintains intracellular Na^+ low, thus providing a favorable gradient to drive the apically located $Na^+-K^+-2Cl^-$ cotransporter (an example of secondary active transport). The apically located renal outer medullary K^+ (ROMK) channel provides a pathway for K^+ to recycle from cell to lumen, and ensures an adequate supply of K^+ to sustain $Na^+-K^+-2Cl^-$ cotransport. This movement through ROMK creates a lumen-positive voltage, providing a driving force for passive K^+ reabsorption through the paracellular pathway. Some of the K^+ entering the cell through the cotransporter exits the cell across the basolateral membrane, accounting for transcellular K^+ reabsorption. K^+ can exit the cell through a conductive pathway or in cotransport with Cl^- . $ClC-Kb$ is the primary pathway for Cl^- efflux across the basolateral membrane.

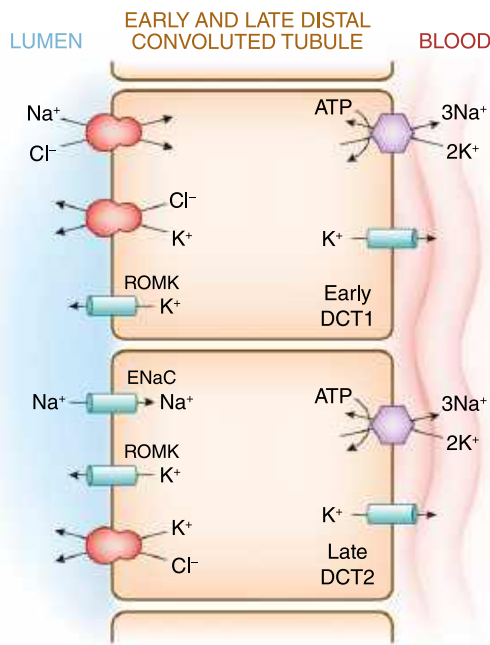


Figure 5. | A cell model for K^+ transport in the distal convoluted tubule (DCT). In the early DCT, luminal Na^+ uptake is mediated by the apically located thiazide-sensitive Na^+-Cl^- cotransporter. The transporter is energized by the basolateral $Na^+-K^+-ATPase$, which maintains intracellular Na^+ concentration low, thus providing a favorable gradient for Na^+ entry into the cell through secondary active transport. The cotransporter is abundantly expressed in the DCT1 but progressively declines along the DCT2. ROMK is expressed throughout the DCT and into the cortical collecting duct. Expression of the epithelial Na^+ channel (ENaC), which mediates amiloride-sensitive Na^+ absorption, begins in the DCT2 and is robustly expressed throughout the downstream connecting tubule and cortical collecting duct. The DCT2 is the beginning of the aldosterone-sensitive distal nephron (ASDN) as identified by the presence of both the mineralocorticoid receptor and the enzyme 11β -hydroxysteroid dehydrogenase II. This enzyme maintains the mineralocorticoid receptor free to only bind aldosterone by metabolizing cortisol to cortisone, the latter of which has no affinity for the receptor. Electrogenic-mediated K^+ transport begins in the DCT2 with the combined presence of ROMK, ENaC, and aldosterone sensitivity. Electroneutral K^+-Cl^- cotransport is present in the DCT and collecting duct. Conditions that cause a low luminal Cl^- concentration increase K^+ secretion through this mechanism, which occurs with delivery of poorly reabsorbable anions, such as sulfate, phosphate, or bicarbonate.

A second principal determinant affecting K^+ secretion is the rate of distal delivery of Na^+ and water. Increased distal delivery of Na^+ stimulates distal Na^+ absorption, which will make the luminal potential more negative and, thus, increase K^+ secretion. Increased flow rates also increase K^+ secretion. When K^+ is secreted in the collecting duct, the luminal K^+ concentration rises, which decreases the diffusion gradient and slows additional K^+ secretion. At higher luminal flow rates, the same amount of K^+ secretion will be diluted by the larger volume such that the rise in luminal K^+ concentration will be less. Thus, increases in the distal delivery of Na^+ and water stimulate K^+ secretion by lowering luminal K^+ concentration and making the luminal potential more negative.

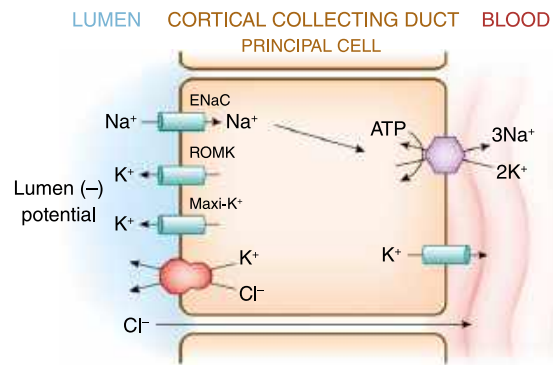


Figure 6. | The cell that is responsible for K^+ secretion in the initial collecting duct and the cortical collecting duct is the principal cell. This cell possesses a basolateral $Na^+-K^+-ATPase$ that is responsible for the active transport of K^+ from the blood into the cell. The resultant high cell K^+ concentration provides a favorable diffusion gradient for movement of K^+ from the cell into the lumen. In addition to establishing a high intracellular K^+ concentration, activity of this pump lowers intracellular Na^+ concentration, thus maintaining a favorable diffusion gradient for movement of Na^+ from the lumen into the cell. Both the movements of Na^+ and K^+ across the apical membrane occur through well defined Na^+ and K^+ channels.

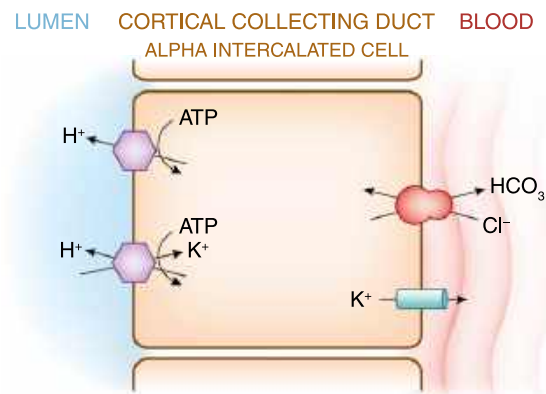


Figure 7. | Reabsorption of HCO_3^- in the distal nephron is mediated by apical H^+ secretion by the α -intercalated cell. Two transporters secrete H^+ , a vacuolar $H^+-ATPase$ and an $H^+-K^+-ATPase$. The $H^+-K^+-ATPase$ uses the energy derived from ATP hydrolysis to secrete H^+ into the lumen and reabsorb K^+ in an electroneutral fashion. The activity of the $H^+-K^+-ATPase$ increases in K^+ depletion and, thus, provides a mechanism by which K^+ depletion enhances both collecting duct H^+ secretion and K^+ absorption.

Two populations of K^+ channels have been identified in the cells of the cortical collecting duct. The renal outer medullary K^+ (ROMK) channel is considered to be the major K^+ -secretory pathway. This channel is characterized by having low conductance and a high probability of being open under physiologic conditions. The maxi- K^+ channel (also known as the large-conductance K^+ [BK] channel) is characterized by a large single channel conductance and quiescence in the basal state and activation under conditions of increased flow (18). In addition to increased delivery of Na^+ and dilution of luminal K^+ concentration, recruitment of maxi- K^+ channels contributes to flow-dependent increased K^+ secretion. Renal K^+ channels are subjects of extensive reviews (19–21).

The effect of increased tubular flow to activate maxi-K⁺ channels may be mediated by changes in intracellular Ca²⁺ concentration (22). The channel is Ca²⁺-activated, and an acute increase in flow increases intracellular Ca²⁺ concentrations in the principal cell. It has been suggested that the central cilium (a structure present in principal cells) may facilitate transduction of signals of increased flow to increased intracellular Ca²⁺ concentration. In cultured cells, bending of primary cilia results in a transient increase in intracellular Ca²⁺, an effect blocked by antibodies to polycystin 2 (23). Although present in nearly all segments of the nephron, the maxi-K channel has been identified as the mediator of flow-induced K⁺ secretion in the distal nephron and cortical collecting duct (24).

Development of hypokalemia in type II Bartter syndrome illustrates the importance of maxi-K⁺ channels in renal K⁺ excretion (25). Patients with type II Bartter syndrome have a loss-of-function mutation in ROMK manifesting with clinical features of the disease in the perinatal period. ROMK provides the pathway for recycling of K⁺ across the apical membrane in the thick ascending limb of Henle. This recycling generates a lumen-positive potential that drives the paracellular reabsorption of Ca²⁺ and Mg²⁺ and provides luminal K⁺ to the Na⁺-K⁺-2Cl⁻ cotransporter (Figure 4).

Mutations in ROMK decrease NaCl and fluid reabsorption in the thick limb, mimicking a loop diuretic effect, which causes volume depletion. Despite the increase in distal Na⁺ delivery, K⁺ wasting is not consistently observed, because ROMK is also the major K⁺-secretory pathway for regulated K⁺ excretion in the collecting duct. In fact, in the perinatal period, infants with this form of Bartter syndrome often exhibit a transient hyperkalemia consistent with loss of function of ROMK in the collecting duct. However, over time, these patients develop hypokalemia as a result of increased flow-mediated K⁺ secretion through maxi-K⁺ channels. Studies in an ROMK-deficient mouse model of type II Bartter syndrome are consistent with this mechanism (26). The transient hyperkalemia observed in the perinatal period is likely related to the fact that ROMK channels are functionally expressed earlier than maxi-K⁺ channels during the course of development.

In this regard, growing infants and children are in a state of positive K⁺ balance, which correlates with growth and increasing cell number. Early in development, there is a limited capacity of the distal nephron to secrete K⁺ because of a paucity of both apically located ROMK and maxi-K⁺ channels. The increase in K⁺-secretory capacity with maturation is initially a result of increased expression of ROMK. Several weeks later, maxi-K⁺ channel expression develops, allowing for flow-mediated K⁺ secretion to occur (reviewed in ref. 27). The limitation in distal K⁺ secretion is channel-specific, because the electrochemical gradient favoring K⁺ secretion, as determined by activity of the Na⁺-K⁺-ATPase and Na⁺ reabsorption, is not limiting. Additionally, increased flow rates are accompanied by appropriate increases in Na⁺ reabsorption and intracellular Ca²⁺ concentrations in the distal nephron, despite the absence of stimulatory effect on K⁺ secretion (28). Activity of the H⁺-K⁺-ATPase, which couples K⁺ reabsorption to H⁺ secretion in intercalated cells, is similar in newborns and adults. K⁺ reabsorption through this pump, combined with decreased expression

of K⁺-secretory channels, helps maintain a state of positive K⁺ balance during somatic growth after birth. These features of distal K⁺ handling by the developing kidney are a likely explanation for the high incidence of nonoliguric hyperkalemia in preterm infants (29).

Another physiologic state characterized by a period of positive K⁺ balance is pregnancy, where approximately 300 mEq K⁺ is retained (30). High circulating levels of progesterone may play a role in this adaptation through stimulatory effects on K⁺ and H⁺ transport by the H⁺-K⁺ α_2 -ATPase isoform in the distal nephron (31).

In addition to stimulating maxi-K⁺ channels, increased tubular flow has been shown to stimulate Na⁺ absorption through the epithelial Na⁺ channel (ENaC) in the collecting duct. This increase in absorption not only is because of increased delivery of Na⁺, but also seems to be the result of mechanosensitive properties intrinsic to the channel. Increased flow creates a shear stress that activates ENaCs by increasing channel open probability (32,33).

It has been hypothesized that biomechanical regulation of renal tubular Na⁺ and K⁺ transport in the distal nephron may have evolved as a response to defend against sudden increases in extracellular K⁺ concentration that occur in response to ingestion of K⁺-rich diets typical of early vertebrates (22). According to this hypothesis, an increase in GFR after a protein-rich meal would lead to an increase in distal flow activating the ENaC, increasing intracellular Ca²⁺ concentration, and activating maxi-K⁺ channels. These events would enhance K⁺ secretion, thus providing a buffer to guard against development of hyperkalemia.

In patients with CKD, loss of nephron mass is counterbalanced by an adaptive increase in the secretory rate of K⁺ in remaining nephrons such that K⁺ homeostasis is generally well maintained until the GFR falls below 15–20 ml/min (34). The nature of the adaptive process is thought to be similar to the adaptive process that occurs in response to high dietary K⁺ intake in normal subjects (35). Chronic K⁺ loading in animals augments the secretory capacity of the distal nephron, and, therefore, renal K⁺ excretion is significantly increased for any given plasma K⁺ level. Increased K⁺ secretion under these conditions occurs in association with structural changes characterized by cellular hypertrophy, increased mitochondrial density, and proliferation of the basolateral membrane in cells in the distal nephron and principal cells of the collecting duct. Increased serum K⁺ and mineralocorticoids independently initiate the amplification process, which is accompanied by an increase in Na⁺-K⁺-ATPase activity.

Aldosterone Paradox

Under conditions of volume depletion, activation of the renin-angiotensin system leads to increased aldosterone release. The increase in circulating aldosterone stimulates renal Na⁺ retention, contributing to the restoration of extracellular fluid volume, but occurs without a demonstrable effect on renal K⁺ secretion. Under condition of hyperkalemia, aldosterone release is mediated by a direct effect of K⁺ on cells in the zona glomerulosa. The subsequent increase in circulating aldosterone stimulates renal K⁺ secretion, restoring the serum K⁺ concentration to normal, but does so without concomitant renal Na⁺ retention.

The ability of aldosterone to signal the kidney to stimulate salt retention without K^+ secretion in volume depletion and stimulate K^+ secretion without salt retention in hyperkalemia has been referred to as the aldosterone paradox (36). In part, this ability can be explained by the reciprocal relationship between urinary flow rates and distal Na^+ delivery with circulating aldosterone levels. Under conditions of volume depletion, proximal salt and water absorption increase, resulting in decreased distal delivery of Na^+ and water. Although aldosterone levels are increased, renal K^+ excretion remains fairly constant, because the stimulatory effect of increased aldosterone is counterbalanced by the decreased delivery of filtrate to the distal nephron. Under condition of an expanded extracellular fluid volume, distal delivery of filtrate is increased as a result of decreased proximal tubular fluid reabsorption. Once again, renal K^+ excretion remains relatively constant in this setting, because circulating aldosterone levels are suppressed. It is only under pathophysiologic conditions that increased distal Na^+ and water delivery are coupled to increased aldosterone levels. Renal K^+ wasting will occur in this setting (37) (Figure 8).

Renal K^+ secretion also remains stable during changes in flow rate resulting from variations in circulating vasopressin. In this regard, vasopressin has a stimulatory effect on renal K^+ secretion (38,39). This kaliuretic property may serve to oppose a tendency to K^+ retention under conditions of antidiuresis when a low-flow rate-dependent fall in distal tubular K^+ secretion might otherwise occur. In contrast, suppressed endogenous vasopressin leads to decreased activity of the distal K^+ -secretory mechanism, thus limiting excessive K losses under conditions of full hydration and water diuresis.

Although the inverse relationship between aldosterone levels and distal delivery of salt and water serves to keep renal K^+ excretion independent of volume status, recent reviews have suggested a more complex mechanism centered on the with no lysine [K] 4 (WNK4) protein kinase in

the distal nephron (40,41). WNK4 is one of four members of a family of serine-threonine kinases each encoded by a different gene and characterized by the atypical placement of the catalytic lysine residue that is present in most other protein kinases. Inactivating mutations in WNK4 lead to development of pseudohypoaldosteronism type II (PHAII; Gordon syndrome). This disorder is inherited in an autosomal dominant fashion and is characterized by hypertension and hyperkalemia (42). Circulating aldosterone levels are low, despite the presence of hyperkalemia. Thiazide diuretics are particularly effective in treating both the hypertension and hyperkalemia (43).

Wild-type WNK4 acts to reduce surface expression of the thiazide-sensitive Na^+-Cl^- cotransporter and also stimulates clathrin-dependent endocytosis of ROMK in the collecting duct (44,45). The inactivating mutation of WNK4 responsible for PHAII leads to increased cotransporter activity and further stimulates endocytosis of ROMK. The net effect is increased $NaCl$ reabsorption combined with decreased K^+ secretion. Mutated WNK4 also enhances paracellular Cl^- permeability caused by increased phosphorylation of claudins, which are tight junction proteins involved in regulating paracellular ion transport (46). In addition to increasing Na^+ retention, this change in permeability further impairs K^+ secretion, because the lumen-negative voltage, which normally serves as a driving force for K^+ secretion, is dissipated.

Because development of hypertension and hyperkalemia resulting from the PHAII-mutated WNK4 protein can be viewed as an exaggerated response to a reduction in extracellular fluid volume (salt retention without increased K^+ secretion), it has been proposed that wild-type WNK4 may act as a molecular switch determining balance between renal $NaCl$ reabsorption and K^+ secretion (45,47). Under conditions of volume depletion, the switch would be altered in a manner reminiscent of the PHAII mutant such that $NaCl$ reabsorption is increased, but K^+ secretion is further

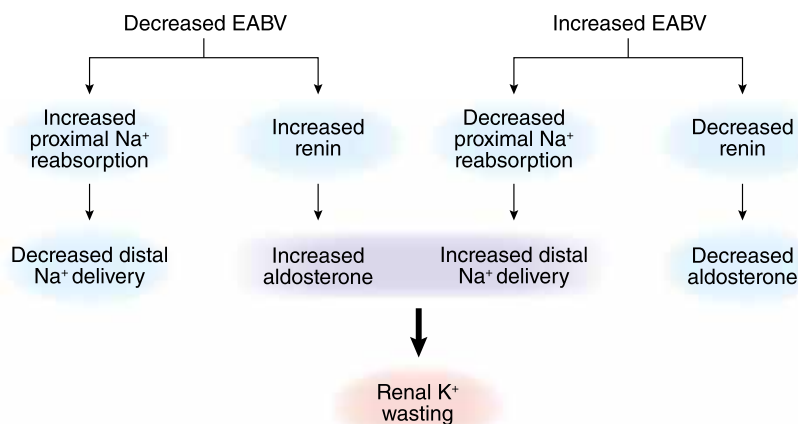


Figure 8. Under normal circumstances, delivery of Na^+ to the distal nephron is inversely associated with serum aldosterone levels. For this reason, renal K^+ excretion is kept independent of changes in extracellular fluid volume. Hypokalemia caused by renal K^+ wasting can be explained by pathophysiologic changes that lead to coupling of increased distal Na^+ delivery and aldosterone or aldosterone-like effects. When approaching the hypokalemia caused by renal K^+ wasting, one must determine whether the primary disorder is an increase in mineralocorticoid activity or an increase in distal Na^+ delivery. EABV, effective arterial blood volume.

inhibited. However, when increased serum K^+ concentration occurs in the absence of volume depletion, WNK4 alterations result in maximal renal K^+ secretion without Na^+ retention.

Angiotensin II (AII) has emerged as an important modulator of this switch. Under conditions of volume depletion, AII and aldosterone levels are increased (Figure 9). In addition to effects leading to enhanced $NaCl$ reabsorption in the proximal tubule, AII activates the Na^+-Cl^- cotransporter in a WNK4-dependent manner, and it is primarily located in the initial part of the distal convoluted tubule (DCT; DCT1) (48,49). AII also activates ENaC, which is found in the aldosterone-sensitive distal nephron (ASDN) comprised of the second segment of the DCT (DCT2), the connecting tubule, and the collecting duct (50). The activation of ENaC by AII is additive to that of aldosterone (51). In this manner, AII and aldosterone act in concert to stimulate Na^+ retention. At the same time, AII inhibits ROMK by both WNK4-dependent and -independent mechanisms (52,53). This inhibitory effect on ROMK along with decreased Na^+ delivery to the collecting duct brought about by AII stimulation of Na^+ reabsorption in the proximal nephron, and DCT1 allows for simultaneous Na^+ conservation without K^+ wasting.

Hyperkalemia, or an increase in dietary K^+ intake, can increase renal K^+ secretion independent of change in mineralocorticoid activity and without causing volume retention. This effect was shown in Wistar rats fed a diet very low in $NaCl$ and K^+ for several days and given a pharmacologic dose of deoxycorticosterone to ensure a constant and nonvariable effect of mineralocorticoids (54,55). After a KCl load administered into the peritoneal cavity, two distinct phases were noted. In the first 2 hours, there was a large increase in the rate of renal K^+ excretion that

was largely caused by an increase in the K^+ concentration in the cortical collecting duct. During this early phase, flow through the collecting duct increased only slightly, suggesting that changes in K^+ concentration were largely caused by an increase in K^+ -secretory capacity of the collecting duct. This effect would be consistent with known effects of dietary supplementation of K^+ to increase channel density of both ROMK and maxi- K^+ channels (56).

In the subsequent 4 hours, renal K^+ excretion continued to be high, but during this second phase, the kaliuresis was mostly accounted for by increased flow through the collecting duct. The increased flow was attributed to an inhibitory effect of increased interstitial K^+ concentration on reabsorption of $NaCl$ in the upstream ascending limb of Henle, an effect supported by microperfusion studies in the past (57,58). The timing of the two phases is presumably important, because higher flows would be most effective in promoting kaliuresis only after establishment of increased channel density. Although older studies are consistent with decreased Na^+ absorption in the thick limb and proximal nephron after increased K^+ intake, inhibitory effects in these high-capacity segments lack the precision and timing necessary to ensure that downstream delivery of Na^+ is appropriate to maximally stimulate K^+ secretion and at the same time, not be excessive, predisposing to volume depletion, particularly in the setting of a low Na^+ diet (57–59).

The low-capacity nature of the DCT and its location immediately upstream from the ASDN make this segment a more likely site for changes in dietary K^+ intake to modulate Na^+ transport and ensure that downstream delivery of Na^+ is precisely the amount needed to ensure maintenance of K^+ homeostasis without causing unwanted effects on volume.

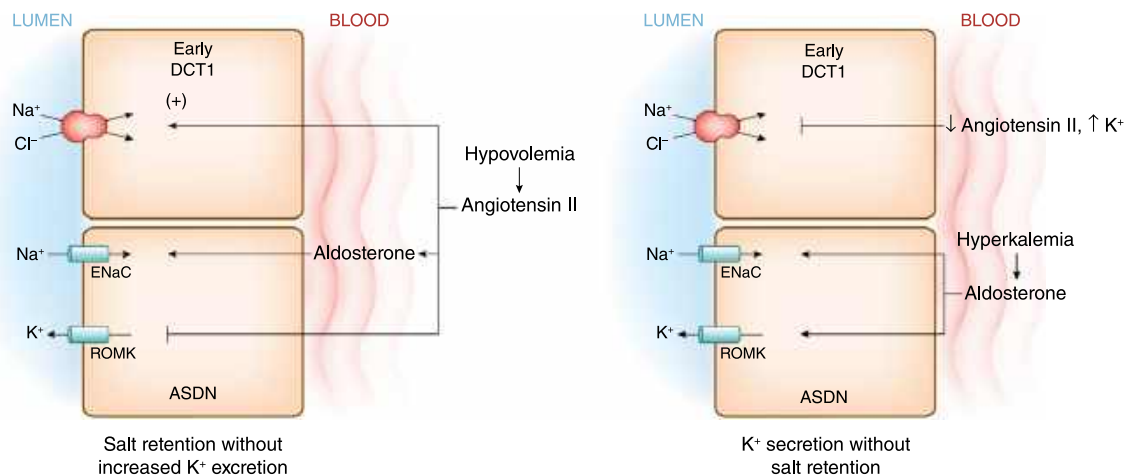


Figure 9. | The aldosterone paradox refers to the ability of the kidney to stimulate $NaCl$ retention with minimal K^+ secretion under conditions of volume depletion and maximize K^+ secretion without Na^+ retention in hyperkalemia. With volume depletion (left panel), increased circulating angiotensin II (AII) levels stimulate the Na^+-Cl^- cotransporter in the early DCT. In the ASDN, AII along with aldosterone stimulate the ENaC. In this latter segment, AII exerts an inhibitory effect on ROMK, thereby providing a mechanism to maximally conserve salt and minimize renal K^+ secretion. When hyperkalemia or increased dietary K^+ intake occurs with normovolemia (right panel), low circulating levels of AII or direct effects of K^+ lead to inhibition of Na^+-Cl^- cotransport activity along with increased activity of ROMK. As a result, Na^+ delivery to the ENaC is optimized for the coupled electrogenic secretion of K^+ through ROMK. As discussed in the text, with no lysine [K] 4 (WNK4) proteins are integrally involved in the signals by which the paradox is brought about. It should be emphasized the WNK proteins are part of a complex signaling network still being fully elucidated. The interested reader is referred to several recent reviews and advancements on this subject (48,51,91–93).

In this regard, increased dietary K^+ intake leads to an inhibitory effect on Na^+ transport in this segment and does so through effects on WNK1, another member of the WNK family of kinases (60,61). WNK1 is ubiquitously expressed throughout the body in multiple spliced forms. By contrast, a shorter WNK1 transcript lacking the amino terminal 1–437 amino acids of the long transcript is highly expressed in the kidney but not other tissues, and it is referred to as kidney-specific WNK1 (KS-WNK1). KS-WNK1 is restricted to the DCT and part of the connecting duct and functions as a physiologic antagonist to the actions of long WNK1. Changes in the ratio of KS-WNK1 and long WNK1 in response to dietary K^+ contribute to the physiologic regulation of renal K^+ excretion (62–65).

Under normal circumstances, long WNK1 prevents the ability of WNK4 to inhibit activity of the Na^+-Cl^- cotransporter in the DCT. Thus, increased activity of long WNK1 leads to a net increase in $NaCl$ reabsorption. Dietary K^+ loading increases the abundance of KS-WNK1. Increased KS-WNK1 antagonizes the inhibitory effect of long WNK1 on WNK4. The net effect is inhibition of Na^+-Cl^- cotransport in the DCT and increased Na^+ delivery to more distal parts of the tubule. In addition, increased KS-WNK1 antagonizes the effect of long WNK1 to stimulate endocytosis of ROMK. Furthermore, KS-WNK1 exerts a stimulatory effect on the ENaC. Thus, increases in KS-WNK1 in response to dietary K^+ loading facilitate K^+ secretion through the combined effects of increased Na^+ delivery through downregulation of Na^+-Cl^- cotransport in the DCT, increased electrogenic Na^+ reabsorption through the ENaC, and greater abundance of ROMK.

Increased aldosterone levels in response to a high K^+ diet lead to effects that complement the effects of KS-WNK1 (66,67). The serum- and glucocorticoid-dependent protein kinase (SGK1) is an immediate transcriptional target of aldosterone binding to the mineralocorticoid receptor. Activation of SGK1 leads to phosphorylation of WNK4, resulting in a loss of the ability of WNK4 to inhibit ROMK and the ENaC (66,68). Aldosterone-induced activation of SGK1 also leads to increased ENaC expression and activity by causing the phosphorylation of ubiquitin protein ligase Nedd4–2. Phosphorylated Nedd4–2 results in less retrieval of ENaC from the apical membrane (69). It should be emphasized that the absence of AII is a critical factor in the ability of high K^+ intake to bring about the changes necessary to facilitate K^+ secretion without excessive Na^+ reabsorption.

Role in Hypertension

Changes in KS-WNK1 and long WNK1 that occur in response to dietary K^+ intake affect renal Na^+ handling in a way that may be of importance in the observed relationship between dietary K^+ intake and hypertension. Epidemiologic studies established that K^+ intake is inversely related to the prevalence of hypertension (70). In addition, K^+ supplements and avoidance of hypokalemia lowers BP in hypertensive subjects. By contrast, BP increases in hypertensive subjects placed on a low K^+ diet. This increase in BP is associated with increased renal Na^+ reabsorption (71).

K^+ deficiency increases the ratio of long WNK1 to KS-WNK1. Long WNK1 is associated with increased retrieval

of ROMK, thus providing an appropriate response to limit K^+ secretion. However, long WNK1 also leads to a stimulatory effect on ENaC activity as well as releasing the inhibitory effect of WNK4 on Na^+ reabsorption mediated by the $NaCl$ cotransporter in the DCT (72,73). These effects suggest that reductions in K^+ secretion under conditions of K^+ deficiency will occur at the expense of increased Na^+ retention.

Renal conservation of K^+ and Na^+ under conditions of K^+ deficiency may be considered an evolutionary adaptation, because dietary K^+ and Na^+ deficiency likely occurred together for early humans (74). However, such an effect is potentially deleterious in our present setting, because evolution has seen a large increase in the ratio of dietary intake of Na^+ versus K^+ . The effects of an increased ratio of WNK1 to KS-WNK1 in the kidney under conditions of modern day high Na^+ /low K^+ diet could be central to the pathogenesis of salt-sensitive hypertension (75).

Enteric Sensor of K^+

There is evidence to support the existence of enteric solute sensors capable of responding to dietary Na^+ , K^+ , and phosphate that signal the kidney to rapidly alter ion excretion or reabsorption (76–78). In experimental animals, and using protocols to maintain identical plasma K^+ concentration, the kaliuretic response to a K^+ load is greater when given as a meal compared with an intravenous infusion (79). These studies suggest that dietary K^+ intake through a splanchnic sensing mechanism can signal increases in renal K^+ excretion independent of changes in plasma K^+ concentration or aldosterone (reviewed in ref. 80).

Although the precise signaling mechanism is not known, recent studies suggest that the renal response may be because of rapid and nearly complete dephosphorylation of the Na^+-Cl^- cotransporter in the DCT, causing decreased activity of the transporter and, thus, enhancing delivery of Na^+ to the ASDN (81,82). In these studies, gastric delivery of K^+ led to dephosphorylation of the cotransporter within minutes independent of aldosterone and based on *in vitro* studies, independent of changes in extracellular K^+ concentration. The temporally associated increase in renal K^+ excretion results from a more favorable electrochemical driving force caused by the downstream shift in Na^+ reabsorption from the DCT to the ENaC in the ASDN as well as increased maxi- K^+ channel K^+ secretion brought on by increased flow. This rapid natriuretic response to increases in dietary K^+ intake is consistent with the BP-lowering effect of K^+ -rich diets discussed earlier.

Circadian Rhythm of K^+ Secretion

During a 24-hour period, urinary K^+ excretion varies in response to changes in activity and fluctuations in K^+ intake caused by the spacing of meals. However, even when K^+ intake and activity are evenly spread over a 24-hour period, there remains a circadian rhythm whereby K^+ excretion is lower at night and in the early morning hours and then increases in the afternoon (83–86). This circadian pattern results from changes in intratubular K^+ concentration in the collecting duct as opposed to variations in urine flow rate (87).

In the mouse distal nephron, a circadian rhythm exists for gene transcripts that encode proteins involving K^+

secretion (88). Gene expression of ROMK is greater during periods of activity, whereas expression of the $H^+K^+-ATPase$ is higher during rest, which correspond to periods when renal K^+ excretion is greater and less, respectively (89). Changes in plasma aldosterone levels may play a contributory role, because circadian rhythm of glucocorticoid synthesis and secretion has been described in the adrenal gland. In addition, expression of clock genes within cells of the distal nephron suggests that a pacemaker function regulating K^+ transport may be an intrinsic component of the kidney that is capable of operating independent of outside influence.

The clinical significance of this rhythmicity in K^+ and other electrolyte secretions is not known. Evidence suggests that dysregulation of circadian rhythms may contribute to a lack of nocturnal decline in BP, with eventual development of sustained hypertension as well as accelerated CKD and cardiovascular disease (85,90).

Disclosures

None.

References

- Palmer BF: A physiologic-based approach to the evaluation of a patient with hyperkalemia. *Am J Kidney Dis* 56: 387–393, 2010
- Foley K, Boguslavsky S, Klip A: Endocytosis, recycling, and regulated exocytosis of glucose transporter 4. *Biochemistry* 50: 3048–3061, 2011
- Ho K: A critically swift response: Insulin-stimulated potassium and glucose transport in skeletal muscle. *Clin J Am Soc Nephrol* 6: 1513–1516, 2011
- Nguyen TQ, Maalouf NM, Sakhaee K, Moe OW: Comparison of insulin action on glucose versus potassium uptake in humans. *Clin J Am Soc Nephrol* 6: 1533–1539, 2011
- Alvestrand A, Wahren J, Smith D, DeFronzo RA: Insulin-mediated potassium uptake is normal in uremic and healthy subjects. *Am J Physiol* 246: E174–E180, 1984
- Williams ME, Gervino EV, Rosa RM, Landsberg L, Young JB, Silva P, Epstein FH: Catecholamine modulation of rapid potassium shifts during exercise. *N Engl J Med* 312: 823–827, 1985
- Clausen T, Nielsen OB: Potassium, Na^+ , K^+ -pumps and fatigue in rat muscle. *J Physiol* 584: 295–304, 2007
- McKenna MJ, Bangsbo J, Renaud JM: Muscle K^+ , Na^+ , and Cl disturbances and Na^+ - K^+ pump inactivation: Implications for fatigue. *J Appl Physiol* (1985) 104: 288–295, 2008
- Clifford PS: Skeletal muscle vasodilatation at the onset of exercise. *J Physiol* 583: 825–833, 2007
- Aronson PS, Giebisch G: Effects of pH on potassium: New explanations for old observations. *J Am Soc Nephrol* 22: 1981–1989, 2011
- Knochel JP, Dotin LN, Hamburger RJ: Pathophysiology of intense physical conditioning in a hot climate. I. Mechanisms of potassium depletion. *J Clin Invest* 51: 242–255, 1972
- McDonough AA, Youn JH: Role of muscle in regulating extracellular $[K^+]$. *Semin Nephrol* 25: 335–342, 2005
- Bundgaard H, Kjeldsen K: Potassium depletion increases potassium clearance capacity in skeletal muscles in vivo during acute repletion. *Am J Physiol Cell Physiol* 283: C1163–C1170, 2002
- Bundgaard H: Potassium depletion improves myocardial potassium uptake in vivo. *Am J Physiol Cell Physiol* 287: C135–C141, 2004
- Velázquez H, Ellison DH, Wright FS: Chloride-dependent potassium secretion in early and late renal distal tubules. *Am J Physiol* 253: F555–F562, 1987
- DuBose TD Jr., Codina J, Burges A, Pressley TA: Regulation of H^+ - K^+ -ATPase expression in kidney. *Am J Physiol* 269: F500–F507, 1995
- Stokes JB: Mineralocorticoid effect on K^+ permeability of the rabbit cortical collecting tubule. *Kidney Int* 28: 640–645, 1985
- Palmer LG, Frindt G: High-conductance K channels in intercalated cells of the rat distal nephron. *Am J Physiol Renal Physiol* 292: F966–F973, 2007
- Hebert SC, Desir G, Giebisch G, Wang W: Molecular diversity and regulation of renal potassium channels. *Physiol Rev* 85: 319–371, 2005
- Wang WH: Regulation of ROMK (Kir1.1) channels: New mechanisms and aspects. *Am J Physiol Renal Physiol* 290: F14–F19, 2006
- Grimm PR, Sansom SC: BK channels in the kidney. *Curr Opin Nephrol Hypertens* 16: 430–436, 2007
- Satlin LM, Carattino MD, Liu W, Kleyman TR: Regulation of cation transport in the distal nephron by mechanical forces. *Am J Physiol Renal Physiol* 291: F923–F931, 2006
- Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33: 129–137, 2003
- Welling PA: Regulation of renal potassium secretion: Molecular mechanisms. *Semin Nephrol* 33: 215–228, 2013
- Pluznick JL, Sansom SC: BK channels in the kidney: Role in $K(+)$ secretion and localization of molecular components. *Am J Physiol Renal Physiol* 291: F517–F529, 2006
- Bailey MA, Cantone A, Yan Q, MacGregor GG, Leng Q, Amorim JB, Wang T, Hebert SC, Giebisch G, Malnic G: Maxi-K channels contribute to urinary potassium excretion in the ROMK-deficient mouse model of Type II Bartter's syndrome and in adaptation to a high-K diet. *Kidney Int* 70: 51–59, 2006
- Gurkan S, Estilo GK, Wei Y, Satlin LM: Potassium transport in the maturing kidney. *Pediatr Nephrol* 22: 915–925, 2007
- Woda CB, Miyawaki N, Ramalakshmi S, Ramkumar M, Rojas R, Zvilowitz B, Kleyman TR, Satlin LM: Ontogeny of flow-stimulated potassium secretion in rabbit cortical collecting duct: Functional and molecular aspects. *Am J Physiol Renal Physiol* 285: F629–F639, 2003
- Thayyil S, Kempley ST, Sinha A: Can early-onset nonoliguric hyperkalemia be predicted in extremely premature infants? *Am J Perinatol* 25: 129–133, 2008
- Lindheimer MD, Richardson DA, Ehrlich EN, Katz AI: Potassium homeostasis in pregnancy. *J Reprod Med* 32: 517–522, 1987
- Elabida B, Edwards A, Salhi A, Azroyan A, Fodstad H, Meneton P, Doucet A, Bloch-Faure M, Crambert G: Chronic potassium depletion increases adrenal progesterone production that is necessary for efficient renal retention of potassium. *Kidney Int* 80: 256–262, 2011
- Morimoto T, Liu W, Woda C, Carattino MD, Wei Y, Hughey RP, Apodaca G, Satlin LM, Kleyman TR: Mechanism underlying flow stimulation of sodium absorption in the mammalian collecting duct. *Am J Physiol Renal Physiol* 291: F663–F669, 2006
- Carattino MD, Sheng S, Kleyman TR: Mutations in the pore region modify epithelial sodium channel gating by shear stress. *J Biol Chem* 280: 4393–4401, 2005
- van Ypersele de Strihou C: Potassium homeostasis in renal failure. *Kidney Int* 11: 491–504, 1977
- Stanton BA: Renal potassium transport: Morphological and functional adaptations. *Am J Physiol* 257: R989–R997, 1989
- Arroyo JP, Ronzaud C, Lagnaz D, Staub O, Gamba G: Aldosterone paradox: Differential regulation of ion transport in distal nephron. *Physiology (Bethesda)* 26: 115–123, 2011
- Palmer BF: A physiologic-based approach to the evaluation of a patient with hypokalemia. *Am J Kidney Dis* 56: 1184–1190, 2010
- Field MJ, Stanton BA, Giebisch G: Influence of ADH on renal potassium handling: A micropuncture and microperfusion study. *Kidney Int* 25: 502–511, 1984
- Cassola AC, Giebisch G, Wang W: Vasopressin increases density of apical low-conductance K^+ channels in rat CCD. *Am J Physiol* 264: F502–F509, 1993
- Kahle KT, Ring AM, Lifton RP: Molecular physiology of the WNK kinases. *Annu Rev Physiol* 70: 329–355, 2008
- Kahle KT, Rinehart J, Giebisch G, Gamba G, Hebert SC, Lifton RP: A novel protein kinase signaling pathway essential for blood pressure regulation in humans. *Trends Endocrinol Metab* 19: 91–95, 2008
- Mayan H, Vered I, Mouallem M, Tzadok-Witkon M, Pauzner R, Farfel Z: Pseudohypoaldosteronism type II: Marked sensitivity to

- thiazides, hypercalciuria, normomagnesemia, and low bone mineral density. *J Clin Endocrinol Metab* 87: 3248–3254, 2002
43. Gordon RD: The syndrome of hypertension and hyperkalemia with normal GFR. A unique pathophysiological mechanism for hypertension? *Clin Exp Pharmacol Physiol* 13: 329–333, 1986
 44. Yang SS, Morimoto T, Rai T, Chiga M, Soharu E, Ohno M, Uchida K, Lin SH, Moriguchi T, Shibuya H, Kondo Y, Sasaki S, Uchida S: Molecular pathogenesis of pseudohypoaldosteronism type II: Generation and analysis of a Wnk4(D561A/+) knockin mouse model. *Cell Metab* 5: 331–344, 2007
 45. Lalioti MD, Zhang J, Volkman HM, Kahle KT, Hoffmann KE, Toka HR, Nelson-Williams C, Ellison DH, Flavell R, Booth CJ, Lu Y, Geller DS, Lifton RP: Wnk4 controls blood pressure and potassium homeostasis via regulation of mass and activity of the distal convoluted tubule. *Nat Genet* 38: 1124–1132, 2006
 46. Yamauchi K, Rai T, Kobayashi K, Soharu E, Suzuki T, Itoh T, Suda S, Hayama A, Sasaki S, Uchida S: Disease-causing mutant WNK4 increases paracellular chloride permeability and phosphorylates claudins. *Proc Natl Acad Sci U S A* 101: 4690–4694, 2004
 47. Kahle KT, Wilson FH, Lifton RP: Regulation of diverse ion transport pathways by WNK4 kinase: A novel molecular switch. *Trends Endocrinol Metab* 16: 98–103, 2005
 48. Castañeda-Bueno M, Gamba G: Mechanisms of sodium-chloride cotransporter modulation by angiotensin II. *Curr Opin Nephrol Hypertens* 21: 516–522, 2012
 49. Castañeda-Bueno M, Cervantes-Pérez LG, Vázquez N, Uribe N, Kantesaria S, Morla L, Bobadilla NA, Doucet A, Alessi DR, Gamba G: Activation of the renal Na⁺:Cl⁻ cotransporter by angiotensin II is a WNK4-dependent process. *Proc Natl Acad Sci U S A* 109: 7929–7934, 2012
 50. Sun P, Yue P, Wang WH: Angiotensin II stimulates epithelial sodium channels in the cortical collecting duct of the rat kidney. *Am J Physiol Renal Physiol* 302: F679–F687, 2012
 51. van der Lubbe N, Zietse R, Hoorn EJ: Effects of angiotensin II on kinase-mediated sodium and potassium transport in the distal nephron. *Curr Opin Nephrol Hypertens* 22: 120–126, 2013
 52. Wei Y, Zamilowicz B, Satlin LM, Wang WH: Angiotensin II inhibits the ROMK-like small conductance K channel in renal cortical collecting duct during dietary potassium restriction. *J Biol Chem* 282: 6455–6462, 2007
 53. Yue P, Sun P, Lin DH, Pan C, Xing W, Wang W: Angiotensin II diminishes the effect of SGK1 on the WNK4-mediated inhibition of ROMK1 channels. *Kidney Int* 79: 423–431, 2011
 54. Halperin ML, Cheema-Dhadli S, Lin SH, Kamel KS: Control of potassium excretion: A Paleolithic perspective. *Curr Opin Nephrol Hypertens* 15: 430–436, 2006
 55. Cheema-Dhadli S, Lin SH, Keong-Chong C, Kamel KS, Halperin ML: Requirements for a high rate of potassium excretion in rats consuming a low electrolyte diet. *J Physiol* 572: 493–501, 2006
 56. Najjar F, Zhou H, Morimoto T, Bruns JB, Li HS, Liu W, Kleyman TR, Satlin LM: Dietary K⁺ regulates apical membrane expression of maxi-K channels in rabbit cortical collecting duct. *Am J Physiol Renal Physiol* 289: F922–F932, 2005
 57. Stokes JB: Consequences of potassium recycling in the renal medulla. Effects of ion transport by the medullary thick ascending limb of Henle's loop. *J Clin Invest* 70: 219–229, 1982
 58. Sufit CR, Jamison RL: Effect of acute potassium load on reabsorption in Henle's loop in the rat. *Am J Physiol* 245: F569–F576, 1983
 59. Brandis M, Keyes J, Windhager EE: Potassium-induced inhibition of proximal tubular fluid reabsorption in rats. *Am J Physiol* 222: 421–427, 1972
 60. Cheng CJ, Truong T, Baum M, Huang CL: Kidney-specific WNK1 inhibits sodium reabsorption in the cortical thick ascending limb. *Am J Physiol Renal Physiol* 303: F667–F673, 2012
 61. Liu Z, Xie J, Wu T, Truong T, Auchus RJ, Huang CL: Down-regulation of NCC and NKCC2 cotransporters by kidney-specific WNK1 revealed by gene disruption and transgenic mouse models. *Hum Mol Genet* 20: 855–866, 2011
 62. O'Reilly M, Marshall E, Macgillivray T, Mittal M, Xue W, Kenyon CJ, Brown RW: Dietary electrolyte-driven responses in the renal WNK kinase pathway in vivo. *J Am Soc Nephrol* 17: 2402–2413, 2006
 63. Wade JB, Fang L, Liu J, Li D, Yang CL, Subramanya AR, Maouyo D, Mason A, Ellison DH, Welling PA: WNK1 kinase isoform switch regulates renal potassium excretion. *Proc Natl Acad Sci U S A* 103: 8558–8563, 2006
 64. Lazrak A, Liu Z, Huang CL: Antagonistic regulation of ROMK by long and kidney-specific WNK1 isoforms. *Proc Natl Acad Sci U S A* 103: 1615–1620, 2006
 65. Cope G, Murthy M, Golbang AP, Hamad A, Liu CH, Cuthbert AW, O'Shaughnessy KM: WNK1 affects surface expression of the ROMK potassium channel independent of WNK4. *J Am Soc Nephrol* 17: 1867–1874, 2006
 66. Ring AM, Leng Q, Rinehart J, Wilson FH, Kahle KT, Hebert SC, Lifton RP: An SGK1 site in WNK4 regulates Na⁺ channel and K⁺ channel activity and has implications for aldosterone signaling and K⁺ homeostasis. *Proc Natl Acad Sci U S A* 104: 4025–4029, 2007
 67. Náray-Fejes-Tóth A, Snyder PM, Fejes-Tóth G: The kidney-specific WNK1 isoform is induced by aldosterone and stimulates epithelial sodium channel-mediated Na⁺ transport. *Proc Natl Acad Sci U S A* 101: 17434–17439, 2004
 68. Lin DH, Yue P, Rinehart J, Sun P, Wang Z, Lifton R, Wang WH: Protein phosphatase 1 modulates the inhibitory effect of With-no-Lysine kinase 4 on ROMK channels. *Am J Physiol Renal Physiol* 303: F110–F119, 2012
 69. Palmer BF, Alpern RJ: Liddle's syndrome. *Am J Med* 104: 301–309, 1998
 70. Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM; American Heart Association: Dietary approaches to prevent and treat hypertension: A scientific statement from the American Heart Association. *Hypertension* 47: 296–308, 2006
 71. Krishna GG, Kapoor SC: Potassium depletion exacerbates essential hypertension. *Ann Intern Med* 115: 77–83, 1991
 72. Vallon V, Wulff P, Huang DY, Loffing J, Völkl H, Kuhl D, Lang F: Role of Sgk1 in salt and potassium homeostasis. *Am J Physiol Regul Integr Comp Physiol* 288: R4–R10, 2005
 73. Rozansky DJ: The role of aldosterone in renal sodium transport. *Semin Nephrol* 26: 173–181, 2006
 74. Eaton SB: The ancestral human diet: What was it and should it be a paradigm for contemporary nutrition? *Proc Nutr Soc* 65: 1–6, 2006
 75. Huang CL, Kuo E: Mechanisms of disease: WNK-ing at the mechanism of salt-sensitive hypertension. *Nat Clin Pract Nephrol* 3: 623–630, 2007
 76. Thomas L, Kumar R: Control of renal solute excretion by enteric signals and mediators. *J Am Soc Nephrol* 19: 207–212, 2008
 77. Michell AR, Debnam ES, Unwin RJ: Regulation of renal function by the gastrointestinal tract: Potential role of gut-derived peptides and hormones. *Annu Rev Physiol* 70: 379–403, 2008
 78. Lee FN, Oh G, McDonough AA, Youn JH: Evidence for gut factor in K⁺ homeostasis. *Am J Physiol Renal Physiol* 293: F541–F547, 2007
 79. Oh KS, Oh YT, Kim SW, Kita T, Kang I, Youn JH: Gut sensing of dietary K⁺ intake increases renal K⁺ excretion. *Am J Physiol Regul Integr Comp Physiol* 301: R421–R429, 2011
 80. Youn JH: Gut sensing of potassium intake and its role in potassium homeostasis. *Semin Nephrol* 33: 248–256, 2013
 81. Sorensen MV, Grossmann S, Roesinger M, Gresko N, Todkar AP, Barmettler G, Ziegler U, Odermatt A, Loffing-Cueni D, Loffing J: Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice. *Kidney Int* 83: 811–824, 2013
 82. McDonough AA, Youn JH: Need to quickly excrete K(+) ? Turn off NCC. *Kidney Int* 83: 779–782, 2013
 83. Firsov D, Tokonami N, Bonny O: Role of the renal circadian timing system in maintaining water and electrolytes homeostasis. *Mol Cell Endocrinol* 349: 51–55, 2012
 84. Firsov D, Bonny O: Circadian regulation of renal function. *Kidney Int* 78: 640–645, 2010
 85. Bonny O, Firsov D: Circadian regulation of renal function and potential role in hypertension. *Curr Opin Nephrol Hypertens* 22: 439–444, 2013
 86. Gumz ML, Rabinowitz L: Role of circadian rhythms in potassium homeostasis. *Semin Nephrol* 33: 229–236, 2013
 87. Steele A, deVeber H, Quaggin SE, Scheich A, Ethier J, Halperin ML: What is responsible for the diurnal variation in potassium excretion? *Am J Physiol* 267: R554–R560, 1994
 88. Zuber AM, Centeno G, Pradervand S, Nikolaeva S, Maquelin L, Cardinaux L, Bonny O, Firsov D: Molecular clock is involved in

- predictive circadian adjustment of renal function. *Proc Natl Acad Sci U S A* 106: 16523–16528, 2009
89. Salhi A, Centeno G, Firsov D, Crambert G: Circadian expression of H,K-ATPase type 2 contributes to the stability of plasma K⁺ levels. *FASEB J* 26: 2859–2867, 2012
 90. Gumz ML, Stow LR, Lynch JJ, Greenlee MM, Rudin A, Cain BD, Weaver DR, Wingo CS: The circadian clock protein Period 1 regulates expression of the renal epithelial sodium channel in mice. *J Clin Invest* 119: 2423–2434, 2009
 91. Wakabayashi M, Mori T, Isobe K, Sohara E, Susa K, Araki Y, Chiga M, Kikuchi E, Nomura N, Mori Y, Matsuo H, Murata T, Nomura S, Asano T, Kawaguchi H, Nonoyama S, Rai T, Sasaki S, Uchida S: Impaired KLHL3-mediated ubiquitination of WNK4 causes human hypertension. *Cell Rep* 3: 858–868, 2013
 92. Shibata S, Zhang J, Puthumana J, Stone KL, Lifton RP: Kelch-like 3 and Cullin 3 regulate electrolyte homeostasis via ubiquitination and degradation of WNK4. *Proc Natl Acad Sci U S A* 110: 7838–7843, 2013
 93. Hoorn EJ, Nelson JH, McCormick JA, Ellison DH: The WNK kinase network regulating sodium, potassium, and blood pressure. *J Am Soc Nephrol* 22: 605–614, 2011

Published online ahead of print. Publication date available at www.cjasn.org.



Renal Control of Calcium, Phosphate, and Magnesium Homeostasis

Judith Blaine, Michel Chonchol, and Moshe Levi

Abstract

Calcium, phosphate, and magnesium are multivalent cations that are important for many biologic and cellular functions. The kidneys play a central role in the homeostasis of these ions. Gastrointestinal absorption is balanced by renal excretion. When body stores of these ions decline significantly, gastrointestinal absorption, bone resorption, and renal tubular reabsorption increase to normalize their levels. Renal regulation of these ions occurs through glomerular filtration and tubular reabsorption and/or secretion and is therefore an important determinant of plasma ion concentration. Under physiologic conditions, the whole body balance of calcium, phosphate, and magnesium is maintained by fine adjustments of urinary excretion to equal the net intake. This review discusses how calcium, phosphate, and magnesium are handled by the kidneys.

Clin J Am Soc Nephrol 10: 1257–1272, 2015. doi: 10.2215/CJN.09750913

Division of Renal Diseases and Hypertension, Department of Medicine, University of Colorado Denver, Aurora, Colorado

Correspondence:

Dr. Judith Blaine, Division of Renal Diseases and Hypertension, Department of Medicine, 12605 E. 16th Avenue, University of Colorado Denver, Aurora, CO 80045. Email: Judith.Blaine@ucdenver.edu

Note: This manuscript was updated August 19, 2015 with revised images for Figures 2, 5, and 6.

Introduction

Imbalances of calcium, phosphorus, and magnesium result in a number of serious clinical complications, including arrhythmias, seizures, and respiratory difficulties. The kidney plays a critical role in regulating serum levels of these ions. Regulation of calcium, phosphate, and magnesium occurs in different parts of the nephron and involves a number of different channels, transporters, and pathways. Below we describe the mechanisms governing renal control of these ions.

Calcium

The total amount of calcium in the human body ranges from 1000 to 1200 g. Approximately 99% of body calcium resides in the skeleton; the other 1% is present in the extracellular and intracellular spaces. Although >99% of the total body calcium is located in bone, calcium is a critical cation in both the extracellular and intracellular spaces. Approximately 1% of the calcium in the skeleton is freely exchangeable with calcium in the extracellular fluid compartment. Serum calcium concentration is held in a very narrow range in both spaces. Calcium serves a vital role in nerve impulse transmission, muscular contraction, blood coagulation, hormone secretion, and intercellular adhesion (1,2).

Gastrointestinal Absorption of Calcium

Calcium balance is tightly regulated by the concerted action of calcium absorption in the intestine, reabsorption in the kidney, and exchange from bone, which are all under the control of the calciotropic hormones that are released upon a demand for calcium (Figure 1A). In healthy adults, approximately 800–1000 mg of calcium should be ingested daily. This amount will vary depending on the amount of dairy product consumed.

When 1 g of calcium is ingested in the diet, approximately 800 mg is excreted in the feces and 200 mg in the urine. Approximately 400 mg of the usual 1000 mg dietary calcium intake is absorbed by the intestine, and calcium loss by way of intestinal secretions is approximately 200 mg/d. Therefore, a net absorption of calcium is approximately 200 mg/d (20%) (3). Although serum calcium levels can be maintained in the normal range by bone resorption, dietary intake is the only source by which the body can replenish stores of calcium in bone. Calcium is absorbed almost exclusively within the duodenum, jejunum, and ileum. Each of these intestinal segments has a high absorptive capacity for calcium, with their relative calcium absorption being dependent on the length of each respective intestinal segment and the transit time of the food bolus (3).

There are two routes for the absorption of calcium across the intestinal epithelium: the paracellular pathway (*i.e.*, between the cells) and the transcellular route (*i.e.*, through the cell) (Figure 2A). The paracellular pathway is passive, and it is the predominant route of calcium absorption when the lumen concentration of calcium is high. The paracellular route is indirectly influenced by calcitriol [$1,25(\text{OH})_2\text{D}$] because it is capable of altering the structure of intracellular tight junctions by activation of protein kinase C, making the tight junction more permeable to the movement of calcium. However, $1,25(\text{OH})_2\text{D}$ mainly controls the active absorption of calcium. Calcium moves down its concentration gradient through a calcium channel into the apical section of the microvilli. Because the luminal concentration of calcium is usually much higher than the intracellular concentration of calcium, a large concentration gradient favors the passive movement of calcium. Calcium is rapidly and reversibly bound to the calmodulin-actin-myosin I complex. Calcium moves to the basolateral area of

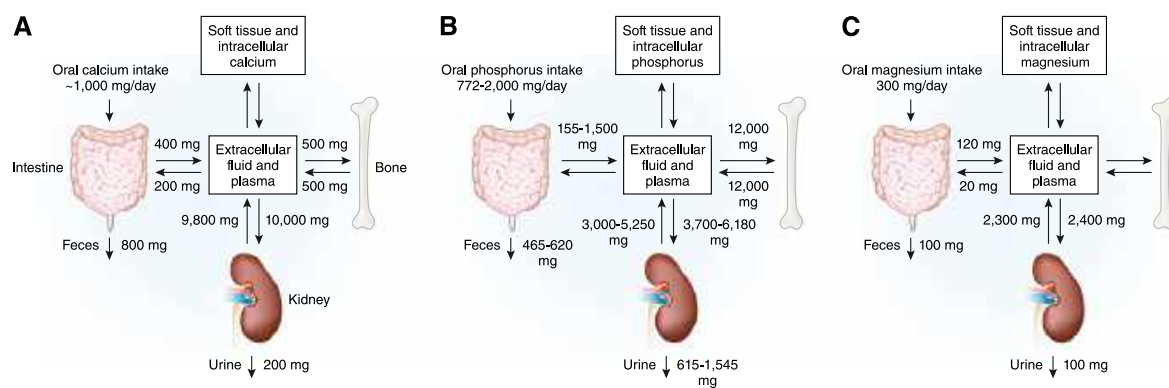


Figure 1. | Calcium, phosphate, and magnesium flux between body compartments. Calcium (A), phosphate (B), and magnesium (C) balance is a complex process involving bone, intestinal absorption of dietary calcium, phosphate, and magnesium, and renal excretion of calcium, phosphate, and magnesium.

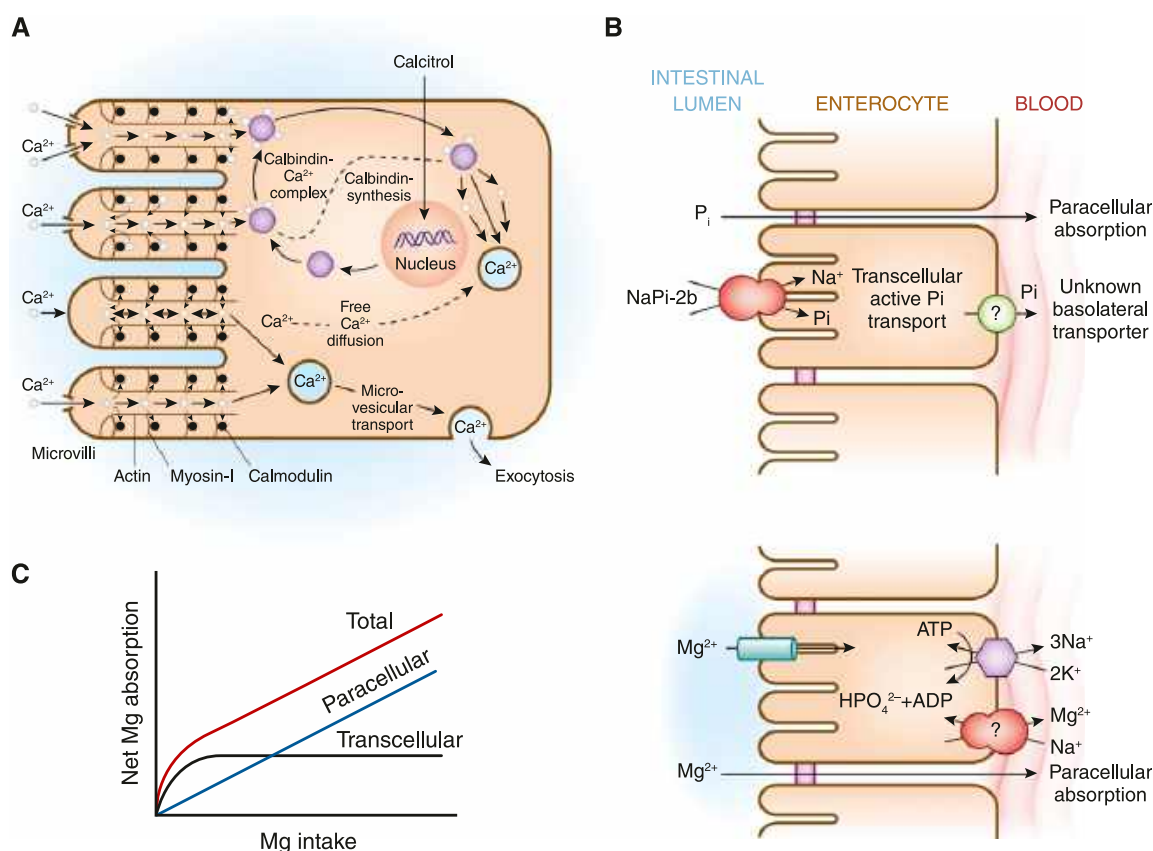


Figure 2. | Intestinal pathways for calcium, phosphorus, and magnesium absorption. (A) Proposed pathways for calcium (Ca) absorption across the intestinal epithelium. Two routes exist for the absorption of Ca across the intestinal epithelium: the paracellular pathway and the transcellular route. (B) Proposed pathways for phosphorus (Pi) absorption across the intestinal epithelium. NaPi2b mediates active transcellular transport of Pi. A paracellular pathway is also believed to exist. (C) Proposed pathways for magnesium (Mg) absorption across the intestinal epithelium. Apical absorption is mediated by the TRPM6/TRPM7 channel, whereas basolateral exit occurs by an Mg exchanger that is yet to be fully defined. A paracellular pathway is also believed to exist. TRPM, transient receptor potential melastatin.

the cell by way of microvesicular transport. As the calmodulin-actin-myosin I complex becomes saturated with calcium, the concentration gradient becomes less favorable, which slows down calcium absorption. 1,25(OH)₂D exerts

influence on the intestinal epithelial cells to increase their synthesis of calbindin. Calcium binds to calbindin, thereby unloading the calcium-calmodulin complexes, which then remove calcium from the microvilli region. This decrease

in calcium concentration again favors the movement of calcium into the microvilli. As the calbindin-calcium complex dissociates, the free intracellular calcium is actively extruded from the cell sodium-calcium (Na-Ca) exchanger (4,5).

Renal Regulation of Calcium Balance

Total serum calcium consists of ionized, protein bound, and complexed fractions (approximately 48%, 46%, and 7%, respectively). The complexed calcium is bound to molecules such as phosphate and citrate. The ultrafilterable calcium equals the total of the ionized and complexed fractions. Normal total serum calcium is approximately 8.9–10.1 mg/dl (about 2.2–2.5 mmol/l). Calcium can be bound to albumin and globulins. For each 1.0-g/dl decrease in serum albumin, total serum calcium decreases by 0.8 mg/dl. For each 1.0-g/dl decrease in serum globulin fraction, total serum calcium decreases by 0.12 mg/dl. Acute alkalosis decreases the ionized calcium. Because both hydrogen ions and calcium are bound to serum albumin, in the presence of metabolic alkalosis, bound hydrogen ions dissociate from albumin, freeing up the albumin to bind with more calcium and thereby decreasing the freely ionized portion of the total serum calcium. For every 0.1 change in pH, ionized calcium changes by 0.12 mg/dl (6).

In humans who have a GFR of 170 liters per 24 hours, roughly 10 g of calcium is filtered per day. The amount of calcium excreted in the urine usually ranges from 100 to 200 mg per 24 hours; hence, 98%–99% of the filtered load of calcium is reabsorbed by the renal tubules. Approximately 60%–70% of the filtered calcium is reabsorbed in the proximal convoluted tubule, 20% in the loop of Henle, 10% by

the distal convoluted tubule, and 5% by the collecting duct. The terminal nephron, although responsible for the reabsorption of only 5%–10% of the filtered calcium load, is the major site for regulation of calcium excretion (1) (Figure 3A).

The reabsorption of calcium in the proximal convoluted tubule parallels that of sodium and water. Proximal tubular calcium reabsorption is thought to occur mainly by passive diffusion and solvent drag. This is based on the observation that the ratio of calcium in the proximal tubule fluid to that in the glomerular filtrate is 1:1.2. The passive paracellular pathways account for approximately 80% of calcium reabsorption in this segment of the nephron. A small but significant component of active calcium transport is observed in the proximal tubules. The active transport of calcium proceeds in a two-step process, with calcium entry from the tubular fluid across the apical membrane and exit through the basolateral membrane. This active transport is generally considered to constitute 10%–15% of total proximal tubule calcium reabsorption and it is mainly regulated by parathyroid hormone (PTH) and calcitonin (7).

No reabsorption of calcium occurs within the thin segment of the loop of Henle (Figure 3A). In the thick ascending limb of the loop of Henle, 20% of the filtered calcium is reabsorbed largely by the cortical thick ascending limb, through both transcellular and paracellular routes. In the thick ascending limb, the bulk of calcium reabsorption proceeds through the paracellular pathway and is proportional to the transtubular electrochemical driving force. The apical $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter NKCC2 and the renal outer medullary potassium K^+ (ROMK) channel

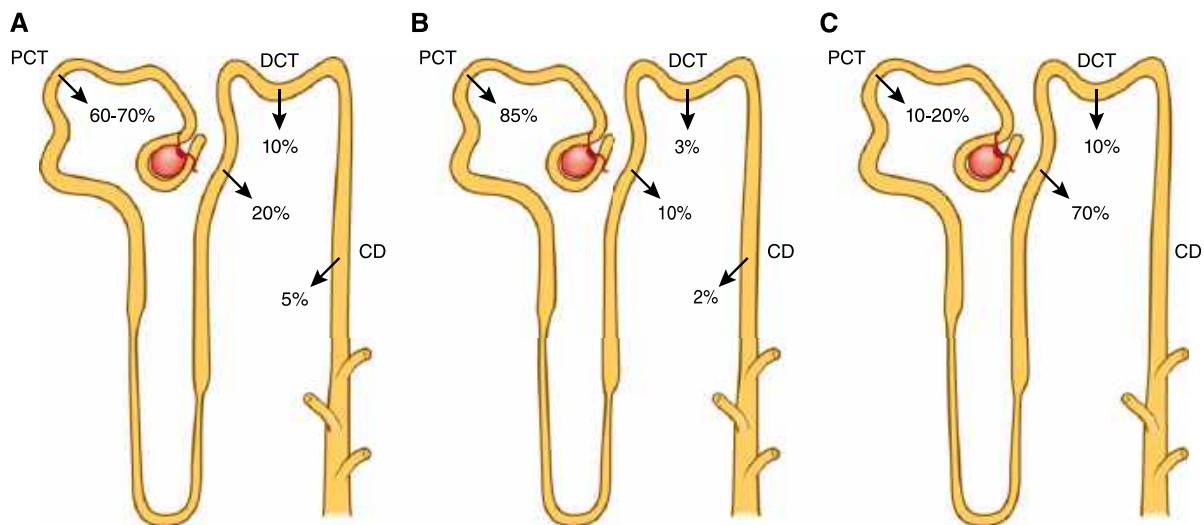


Figure 3. | Schematic illustration of the reabsorption of calcium, phosphorus, and magnesium by different segments of the nephron. (A) Calcium is filtered at the glomerulus, with the ultrafilterable fraction of plasma calcium entering the proximal tubule. Within the proximal convoluted tubule and the proximal straight tubule, 60%–70% of the filtered calcium has been reabsorbed. No reabsorption of calcium occurs within the thin segment of the loop of Henle. The cortical segments of the loop of Henle reabsorb about 20% of the initially filtered load of calcium. Approximately 10% of the filtered calcium is reabsorbed in the distal convoluted tubule, with another 3%–10% of filtered calcium reabsorbed in the connecting tubule. (B) The majority (approximately 85%) of phosphate reabsorption occurs in the proximal convoluted tubule. Approximately 10% of P_i reabsorption occurs in the loop of Henle, 3% occurs in the distal convoluted tubule, and 2% in the collecting duct *via* unidentified pathways. (C) Approximately 10%–30% of the filtered magnesium is absorbed in the proximal tubule, 40%–70% of filtered magnesium is absorbed in the thick ascending limb, and the remaining 5%–10% of magnesium is reabsorbed in the distal convoluted tubule. CD, collecting duct; DCT, distal convoluted tubule; PCT, proximal convoluted tubule.

generate the “driving force” for paracellular cation transport. Whereas NaCl reabsorption through NKCC2 is electroneutral (NKCC2 translocates one Na^+ , one K^+ , and two Cl^- ions from the lumen into the cell), apical potassium represents the rate-limiting step of this process and potassium ions back-diffuse into the lumen through the ROMK channels. Na^+ and Cl^- accumulated inside the cell are then transported into the bloodstream through basolateral $\text{Na}^+-\text{K}^+-\text{ATPase}$ and Cl^- channels, respectively. Overall, these processes yield a net cellular reabsorption of NaCl and the generation of a lumen-positive transepithelial potential difference, which drives nonselective calcium reabsorption through the paracellular route (8) (Figure 4). Calcium transport in the thick ascending limb of the loop of Henle is also influenced by the calcium-sensing receptor (CaSR) (7–10), which is localized in the basolateral membrane. How CaSR controls the calcium reabsorption in the thick ascending limb is now better understood. Using microdissected, *in vitro* microperfused rat cortical thick ascending limb, Loupy *et al.*

showed that an acute inhibition of the CaSR does not alter NaCl reabsorption or the transepithelial potential difference but increased the permeability to calcium of the paracellular pathway (11). The tight junction in the thick ascending limb expresses several claudins, including claudin-14, claudin-16, and claudin-19. A normal expression of claudin-16 and claudin-19 is required for a normal absorption of divalent cations in this tubular segment. Toka *et al.* reported that the disruption of CaSR decreases the abundance of the claudin-14 mRNA and increases that of the claudin-16 mRNA (12). A treatment by cinacalcet increases the abundance of claudin-14 mRNA, and in cell culture models overexpression of claudin-14, decreases the paracellular permeability to calcium (8,12). Calcitropic hormones, such as PTH and calcitonin, stimulate active cellular calcium absorption in the cortical thick ascending limb (7).

In contrast with the proximal tubule and the thick ascending limb of the loop of Henle, the distal tubule reabsorbs calcium exclusively *via* the transcellular route. The distal convoluted tubule absorbs 5%–10% of the

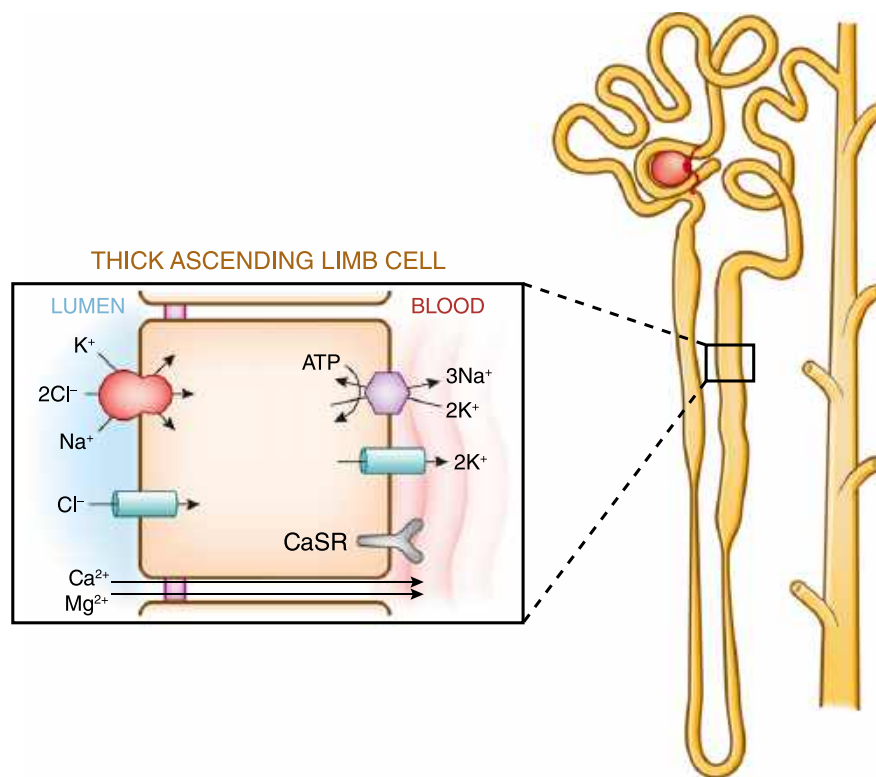


Figure 4. | Model of calcium and magnesium absorption by the thick ascending limb of Henle. Calcium absorption proceeds through both an active, transcellular pathway and by a passive paracellular pathway. Only transport pathways relevant to calcium absorption are shown. Basal absorption is passive and is driven by the ambient electrochemical gradient for calcium. The apical $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransporter and the renal outer medullary potassium K^+ channel generate the “driving force” for paracellular cation transport. Calcitropic hormones, such as parathyroid hormone and calcitonin, stimulate active calcium absorption in cortical thick ascending limbs. Inhibition of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransport by loop diuretics or in Bartter’s syndrome decreases the transepithelial voltage, thus diminishing passive calcium absorption. In the model of magnesium absorption by thick ascending limb of Henle, 40%–70% of filtered magnesium is absorbed in the thick ascending limb by a paracellular pathway, mostly enhanced by lumen-positive transepithelial voltage. The apical $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransporter mediates apical absorption of Na, K, and Cl. The apical renal outer medullary K channel mediates apical recycling of K back to the tubular lumen and generates lumen-positive voltage. Cl channel Kb mediates Cl exit through the basolateral membrane. Here $\text{Na}^+-\text{K}^+-\text{ATPase}$ also mediates Na exit through the basolateral membrane and generates the Na gradient for Na absorption. The tight junction proteins claudin-16 and claudin-19 play a prominent role in magnesium absorption. The calcium-sensing receptor was also recently determined to regulate magnesium transport in this segment: upon stimulation, magnesium transport is decreased. CaSR, calcium-sensing receptor.

filtered calcium. Calcium absorption in this segment is active because it proceeds against a chemical and an electrical gradient. This active process can be divided into three steps. The first step requires calcium influx across the apical membrane. The transient receptor potential vanilloid 5 has been identified as the responsible protein in this process. The second step is the diffusion of calcium through the cytosol. During this process, calbindin-D28k binds intracellular calcium transported *via* transient receptor potential vanilloid 5 and shuttles it through the cytosol toward the basolateral membrane where calcium is extruded *via* sodium-calcium exchanger NCX1 and the plasma membrane calcium-ATPase PMCA1b, which is the final step in this process (13–15). Figure 5 is a cell model of the three-step process of transcellular calcium transport.

Hormonal and Other Factors Regulating Renal Calcium Handling

PTH. Many physiologic, pharmacologic, and pathologic factors influence renal calcium absorption (Table 1). The

most important regulator is PTH, which stimulates calcium absorption. PTH is a polypeptide secreted from the parathyroid gland in response to a decrease in the plasma concentration of ionized calcium. Therefore, the major physiologic role of the parathyroid gland is to regulate calcium homeostasis. PTH acts to increase the plasma concentration of calcium in three ways: (1) it stimulates bone resorption, (2) it enhances intestinal calcium and phosphate absorption by promoting the formation within the kidney of $1,25(\text{OH})_2\text{D}$, and (3) it augments active renal calcium absorption. These effects are reversed by small changes in the serum calcium concentration that lower PTH secretion.

PTH secretion is tightly regulated on a transcriptional and post-transcriptional level dependent on the concentration of extracellular calcium. In fact, PTH gene transcription is increased by hypocalcemia, glucocorticoids, and estrogen. Hypercalcemia also can increase the intracellular degradation of PTH (16). PTH release is increased by hypocalcemia, adrenergic agonists, dopamine, and prostaglandin E_2 (16). Changes in serum calcium are sensed by

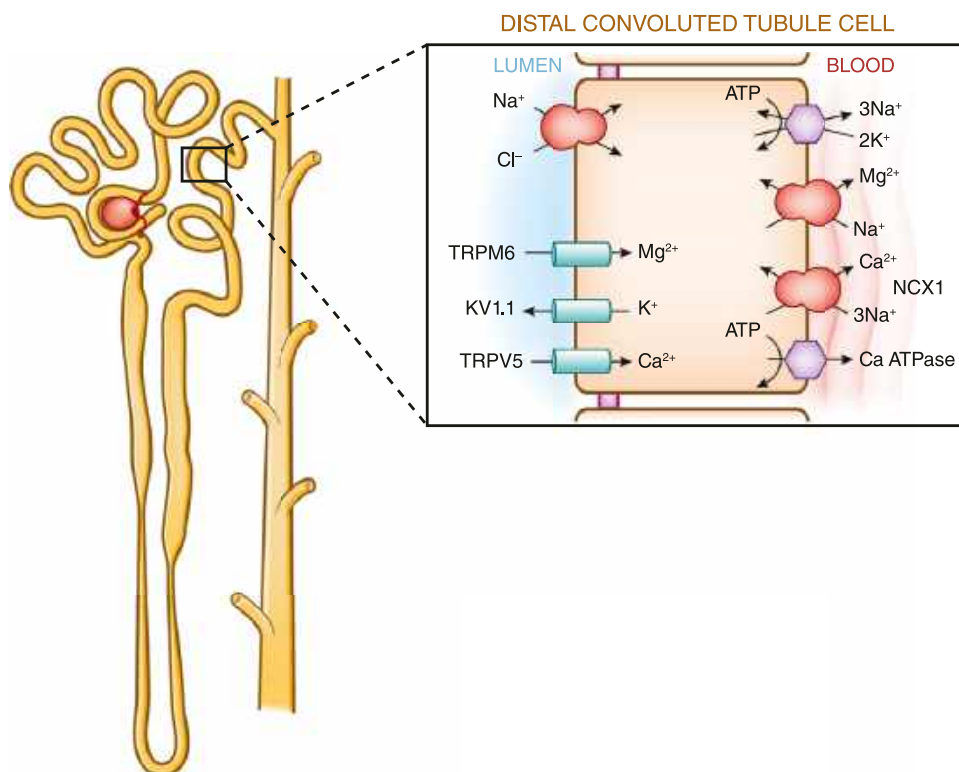


Figure 5. | Model of calcium and magnesium absorption by distal convoluted tubules. Calcium entry across the plasma membrane proceeds through calcium channels with basolateral exit occurring through a combination of the plasma membrane ATPase and $\text{Na}^+-\text{Ca}^{2+}$ exchanger. Calcium absorption is entirely transcellular. Calcitropic hormones such as parathyroid hormone and calcitonin stimulate calcium absorption. Calcitriol [$1,25(\text{OH})_2\text{D}$] stimulates calcium absorption through the activation of nuclear transcription factors. Inhibition of the apical NaCl cotransporter by thiazide diuretics or in Gitelman's syndrome indirectly stimulates calcium absorption. In the model of magnesium absorption by distal convoluted tubules, approximately 5%–10% of magnesium is reabsorbed in the distal convoluted tubule mainly by active transcellular transport mediated by TRPM6. The absorbed magnesium is then extruded *via* a recently identified magnesium/sodium exchanger across the basolateral membrane. The apical K channel $\text{Kv}1.1$ potentiates TRPM6-mediated magnesium absorption by establishing favorable luminal potential. In addition, the basolateral K channel $\text{Kir}4.1$ and the γ -subunit of Na,K-ATPase also regulate magnesium reabsorption.

Table 1. Factors that alter renal regulation of calcium

Increase Calcium Absorption	Decrease Calcium Absorption
Hyperparathyroidism	Hypoparathyroidism
Calcitriol	Low calcitriol levels
Hypocalcemia	Hypercalcemia
Volume contraction	Extracellular fluid expansion
Metabolic alkalosis	Metabolic acidosis
Thiazides diuretics	Loop diuretics

the CaSR, which is localized in the cell membrane of the parathyroid cells. The receptor permits variations in the plasma calcium concentration to be sensed by the parathyroid gland, leading to desired changes in PTH secretion (16).

Vitamin D. Vitamin D₃ (cholecalciferol) is a fat-soluble steroid that is present in the diet and also can be synthesized in the skin from 7-dehydrocholesterol in the presence of ultraviolet light. The hepatic enzyme 25-hydroxylase catalyzes the hydroxylation of vitamin D at the 25 position, resulting in the formation of 25-hydroxyvitamin D or calcidiol. 25-Hydroxyvitamin D produced by the liver enters the circulation and travels to the kidney, bound to vitamin D binding protein. In the kidney, tubular cells contain two enzymes (1 α -hydroxylase and 24 α -hydroxylase) that can further hydroxylate calcidiol, producing 1,25(OH)₂D, the most active form of vitamin D, or 24,25-dihydroxyvitamin D, an inactive metabolite (17). Hence, the vitamin D hormonal system consists of multiple forms, ranging from cutaneous precursors or dietary components to the most active metabolite, 1,25(OH)₂D, which acts upon the target organ receptors to maintain calcium homeostasis and bone health. However, the serum concentration of 25(OH)D, which is the precursor form of the biologically active vitamin D, is the best indicator of the overall vitamin D storage or status (18). The 1,25(OH)₂D enters the circulation and is transported to the small intestine, where it enhances intestinal calcium absorption. The most important endocrine effect of 1,25(OH)₂D in the kidney is a tight control of its own homeostasis through simultaneous suppression of 1 α -hydroxylase and stimulation of 24-hydroxylase. An intact 1,25(OH)₂D–vitamin D receptor system is critical for both basal and PTH-induced osteoclastogenesis. Mature osteoclasts release calcium and phosphorus from the bone, maintaining the appropriate levels of the two minerals in the plasma (17,18).

Serum Calcium. Hypercalcemia is associated with an increase in urinary calcium excretion as a consequence of an increase in the filtered load and a decrease in the tubular reabsorption of calcium. Although hypercalcemia can decrease GFR by renal vasoconstriction, which tends to offset the increase in filtered load, hypercalcemia also causes a decline in tubular reabsorption of calcium by both PTH-dependent and -independent effects. Hypocalcemia decreases renal calcium excretion by decreasing the filtered load and enhancing the tubular reabsorption of calcium (1,17).

Extracellular Fluid. Expansion of the extracellular fluid is associated with an increase in sodium, chloride, and

calcium excretion, whereas reciprocal effects are seen with volume contraction. The mechanisms of this effect are interrelated with the effects of sodium reabsorption and compensatory changes that occur as a result of volume expansion (1).

Metabolic Acidosis. Acute and chronic metabolic acidosis can be associated with an increase in calcium excretion, independent of PTH changes. The calciuria may, in part, be due to the mobilization of calcium from bone, as the hydrogen ion is buffered in the skeleton; however, direct effects of acidosis on tubular calcium resorption also play a role (1,7).

Diuretics. Loop diuretics decrease calcium absorption as a result of inhibition of the transport of sodium chloride at the NKCC2 transporter in the ascending loop of Henle. Thiazide diuretics, which act in the distal tubule, are associated with hypocalciuria (1,7). Two main mechanisms have been proposed to explain the effect of thiazides on calcium excretion: (1) increased proximal sodium and water reabsorption due to volume depletion, and (2) increased distal calcium reabsorption at the thiazide-sensitive site in the distal convoluted tubule.

Clinical Consequences of Alterations in Calcium Balance

Hypocalcemia. The main causes of hypocalcemia can be categorized as follows: (1) lack of PTH (*e.g.*, hereditary or acquired hypoparathyroidism), (2) lack of vitamin D (*e.g.*, dietary deficiency or malabsorption, inadequate sunlight, and defective metabolism such as in liver and kidney disease), and (3) increased calcium complexation (*e.g.*, bone hunger syndrome, rhabdomyolysis, acute pancreatitis, tumor lysis syndrome) (19). Low serum calcium stimulates both PTH synthesis and release. Both hypocalcemia and PTH increase the activity of the 1 α -hydroxylase enzyme in the proximal tubular cells of the nephron, which increases the synthesis of 1,25(OH)₂D. PTH increases bone resorption by osteoclast. In addition, PTH and 1,25(OH)₂D stimulate calcium reabsorption in the distal convoluted tubule. 1,25(OH)₂D increases the fractional absorption of dietary calcium by the gastrointestinal tract. All of these mechanisms aid in returning the serum calcium to normal levels.

Hypercalcemia. Important causes of hypercalcemia include excess PTH production (*e.g.*, primary hyperparathyroidism), excess 1,25(OH)₂D (*e.g.*, vitamin D intoxication, sarcoidosis), increased bone resorption (*e.g.*, metastatic osteolytic tumors, humoral hypercalcemia, immobilization, Paget's disease), increased intestinal absorption of calcium (*e.g.*, milk-alkali syndrome), decreased renal excretion of calcium (*e.g.*, thiazides), and impaired bone formation (*e.g.*, adynamic bone disease). Elevated serum calcium inhibits PTH synthesis and release. Decreases in PTH and hypercalcemia are known to decrease the activity of the 1 α -hydroxylase enzyme, which, in turn, decrease the synthesis of 1,25(OH)₂D. Hypercalcemia also stimulates the C cells in the thyroid gland that increase synthesis of calcitonin. Bone resorption by osteoclasts is blocked by the increased calcitonin and decreased PTH. The decrease in 1,25(OH)₂D decreases gastrointestinal tract absorption of dietary calcium. Low levels of PTH and 1,25(OH)₂D also inhibit calcium reabsorption in the distal convoluted tubule, which increases renal calcium excretion. Finally, in the setting of hypercalcemia, activation of the basolateral CaSR inhibits ROMK

channels, which are important contributors to the potassium recycling into the lumen of the thick ascending limb of the loop of Henle. This effect of hypercalcemia limits the rate of $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport by decreasing the availability of luminal K^+ and therefore, dissipating the lumen-positive transepithelial voltage. The end result is that the CaSR activation diminishes paracellular sodium, calcium, and magnesium transport, producing a phenotype similar to Bartter's syndrome. The signaling pathways underpinning the inhibitory effects of CaSR activation on NKCC2 and ROMK activities involve, at least in part, production of cytochrome P450 metabolites and/or of prostaglandins (8–10). All of these effects tend to return serum calcium to normal levels. Main therapy for hypercalcemia includes saline and loop diuretics that increase renal excretion of calcium and bisphosphonates, which inhibit bone resorption (19).

Phosphorus

Daily Phosphorus Balance

At steady state, oral phosphorus intake is balanced by phosphate (Pi) excretion in the urine and feces (Figure 1B). Daily phosphorus intake varies between 700 and 2000 mg, depending on consumption of phosphorus-rich foods, such as dairy products. After absorption, phosphorus is transported across cell membranes as phosphate (31 mg/1 elemental phosphorus=1 mmol/1 phosphate). Phosphate in the plasma or extracellular fluid undergoes one of three fates: transport into cells, deposition in bone or soft tissue, or elimination predominantly by the kidneys. Within the body, the majority of phosphorus stores are in the bone. Although serum phosphate (Pi) levels constitute <1% of total body phosphorus stores, maintenance of serum Pi within a relatively narrow range (2.5–4.5 mg/dl in adulthood) is crucial for several important cellular processes, including energy metabolism, bone formation, signal transduction, or as a constituent of phospholipids and nucleic acids (20). Maintenance of serum phosphate within the normal range depends on a complex interplay between absorption of phosphate in the gut, exchange with bone stores, shifts between intracellular and intravascular compartments, and renal excretion.

Gastrointestinal Absorption of Phosphate

Although the kidneys are the major regulators of phosphate homeostasis, serum levels of phosphate are also altered by intestinal Pi absorption mediated by the type IIb NaPi cotransporters Npt2b (21) (Figure 2B). Npt2b is regulated by dietary phosphate intake as well as $1,25(\text{OH})_2\text{D}$. In the rat, phosphate reabsorption is greatest in the duodenum and the jejunum, with very little occurring in the ileum. By contrast, in the mouse, Pi is absorbed along the entire intestine with the highest levels of Pi reabsorption occurring in the ileum. The human pattern of intestinal phosphate reabsorption is thought to resemble that found in the rat.

Renal Regulation of Phosphate Balance

The kidney plays a key role in phosphate homeostasis. In normal adults, between 3700 and 6100 mg/d of phosphorus is filtered by the glomerulus (Figure 1B). Net renal excretion of phosphorus is between 600 and 1500 mg/d,

which means that between 75% and 85% of the daily filtered load is reabsorbed by the renal tubules.

Maintenance of normal serum phosphorus levels is primarily achieved through a tightly regulated process of Pi reabsorption from the glomerular filtrate. Within the nephron, approximately 85% of phosphate reabsorption occurs within the proximal tubule (Figure 3B). The remainder of the nephron plays a minor role in Pi regulation and the transporters involved have yet to be identified (22).

Within the proximal tubule, Pi transport from the ultrafiltrate across the proximal tubule epithelium is an energy-dependent process that requires sodium (23). The three renal sodium phosphate cotransporters, Npt2a, Npt2c, and PiT-2, are all positioned in the apical brush border membrane of renal proximal tubule cells and use the energy derived from the transport of sodium down its gradient to move inorganic phosphate from the luminal filtrate into the cell (Figure 6). The amount of phosphate reabsorbed from the filtrate is determined by the abundance of the cotransporters in the apical membrane of proximal tubule cells and not by any alterations in the rate or affinity of Pi transport by post-transcriptional modifications (23). Thus, hormones or dietary factors that alter phosphate reabsorption in the kidney do so by changing the abundance of the sodium phosphate cotransporters in the apical membrane of renal proximal tubule cells. An increase in the brush border levels of the sodium phosphate cotransporters abundance results in increased phosphate absorption from the urine, whereas a decrease in cotransporter abundance leads to phosphaturia. Transport of Pi from the renal proximal tubule to the peritubular capillaries occurs *via* an unknown basolateral transporter (24).

Npt2a and Npt2c are encoded by members of the SLC34 solute carrier family (25), of which Npt2a is encoded by SLC34A1 (26), whereas Npt2c is encoded by SLC34A3 (27). PiT-2 (SLC20A2) is encoded by a member of the SLC20 family (28). Within the proximal tubule in rats and mice, the abundance of Npt2a gradually decreases along the proximal tubule, whereas Npt2c and PiT-2 are expressed mainly in the first (S1) segment (29). Work from knockout studies in rodents has shown that in mice, Npt2a is responsible for the majority (approximately 70%) of the renal regulation of phosphate transport (29). By contrast, linkage analyses have found that in humans, Npt2a and Npt2c may contribute equally to phosphate reabsorption (30,31). The importance of PiT-2 in the renal control of Pi in humans remains unclear (20).

There are a number of functional differences between the three cotransporters (Figure 6). Both Npt2a and Npt2c preferentially transport divalent phosphate. However, Npt2a is electrogenic, transporting three sodium ions into the cell for every one phosphate ion, whereas Npt2c is electroneutral, transporting two sodium ions for every one phosphate ion (32,33). PiT-2, on the other hand, although electrogenic like NaPi2a, preferentially transports monovalent phosphate (23). Changes in pH also affect the transporters differently. Npt2a and Npt2c show a doubling of Pi transport between pH 6.5 and 8, whereas the transport rate of PiT-2 is constant over this range (34,35).

Renal control of phosphate reabsorption is regulated by a number of hormonal and metabolic factors (Table 2) that are discussed below in more detail. These factors change

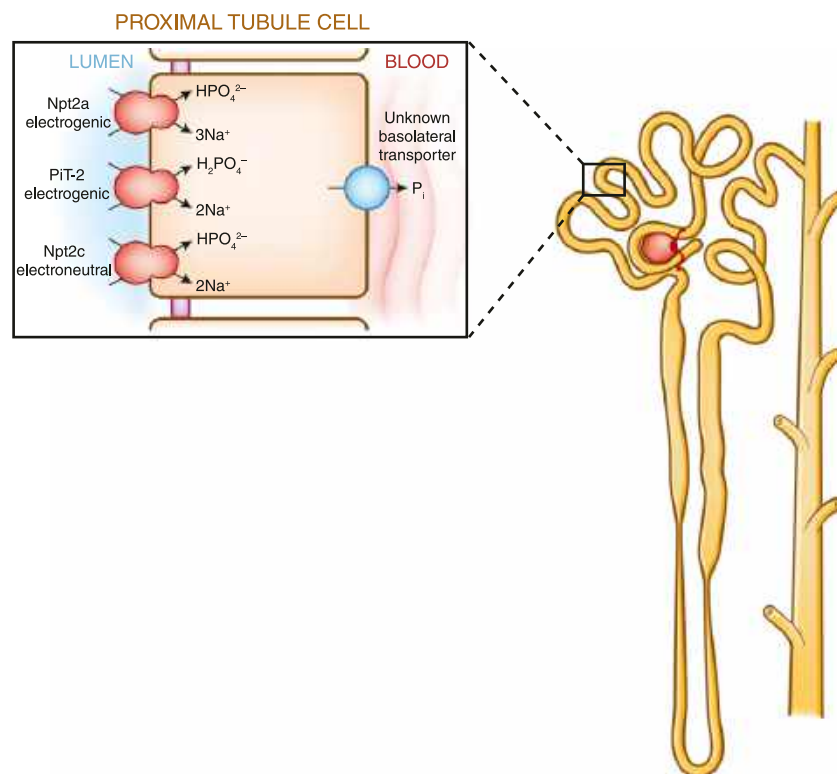


Figure 6. | Model of phosphate reabsorption in the renal proximal tubule. Pi is reabsorbed via three sodium phosphate cotransporters: Npt2a, Npt2c and PiT-2. In humans, Npt2a and Npt2c are believed to play the most important role in phosphate reabsorption. The sodium phosphate cotransporters, which are positioned in the apical membrane of renal proximal tubule cells, use energy derived from the movement of sodium down its gradient to move Pi from the filtrate to the cell interior. The amount of phosphate reabsorbed is dependent on the abundance of the sodium phosphate cotransporters in the apical brush border membrane and hormones such as parathyroid hormone and fibroblast growth factor-23 decrease Pi reabsorption by decreasing the abundance of the sodium phosphate cotransporters in the brush border. Movement of phosphate from the interior of renal proximal tubular cells to the peritubular capillaries occurs via an unknown transporter.

phosphate reabsorption from the ultrafiltrate by changing the abundance of the three sodium phosphate cotransporters in the brush border membrane of the proximal tubule. It should be noted that the time course of response to various stimulatory or inhibitory factors differs between the three cotransporters. In general, dietary or hormonal changes result in relatively rapid (minutes to hours) insertion or removal of Npt2a from the brush border membrane, whereas regulation of Npt2c and PiT-2 occurs more slowly (hours to days) (22).

Dietary Factors Regulating Renal Phosphate Handling

In animals with normal renal function, ingestion of phosphorus-containing foods leads to removal of Npt2a, Npt2c, and PiT-2 from the proximal tubule brush border membrane, thereby decreasing phosphate reabsorption from the ultrafiltrate. By contrast, dietary Pi restriction leads to insertion of the sodium phosphate cotransporters in the proximal tubule brush border membrane, increasing phosphate reabsorption.

Potassium deficiency leads to an increase in phosphate excretion in the urine despite a paradoxical increase in the abundance of Npt2a in the proximal tubule brush border membrane that should increase phosphate reabsorption. Potassium deficiency leads to changes in the brush border

membrane lipid composition that are thought to inhibit Npt2a activity (36).

Hormonal and Other Factors Regulating Renal Phosphate Handling

PTH. PTH causes decreased renal reabsorption of phosphate and phosphaturia by decreasing the abundance of Npt2a, Npt2c, and PiT-2 in the renal proximal tubule brush border membrane (Figure 7). In response to PTH, Npt2a is removed rapidly (within minutes), whereas the decrease in apical membrane abundance of Npt2c and PiT-2 takes hours (27,37,38). The sodium phosphate cotransporter response to PTH involves several kinases, including protein kinases A and C and mitogen-activated protein kinase extracellular signal-regulated kinase 1/2, as well as a myosin motor (myosin VI) (39–41).

Fibroblast Growth Factor-23. Fibroblast growth factor-23 (FGF23) is produced in osteoblasts in response to increases in serum Pi. To exert its physiologic effects on the proximal tubule, FGF23 requires the presence of a cofactor, Klotho, which is produced in the kidney and activates FGF receptor 1 (42). FGF23 reduces the expression and activity of the sodium phosphate cotransporters in the renal proximal tubule and is also thought to decrease the activity of the intestinal sodium phosphate cotransporter. FGF23 also reduces serum

Table 2. Factors that alter renal regulation of phosphate	
Increase Phosphate Absorption	Decrease Phosphate Absorption
Low-phosphate diet 1,25-Vitamin D ₃ Thyroid hormone	Parathyroid hormone Phosphatonins (<i>e.g.</i> , FGF23) High-phosphate diet Metabolic acidosis Potassium deficiency Glucocorticoids Dopamine Hypertension Estrogen
FGF23, fibroblast growth factor-23.	

levels of calcitriol by decreasing the renal expression of 1 α -hydroxylase, which is the rate-limiting step in calcitriol synthesis, and increasing renal expression of 24-hydroxylase, which is required for calcitriol degradation (43) (Figure 8). In addition, FGF23 suppresses PTH synthesis, although the parathyroid glands are believed to become resistant to FGF23 as kidney disease progresses.

1,25(OH)₂D. Calcitriol is believed to increase phosphate reabsorption in the proximal tubule (44) but the effects are confounded by the fact that changes in 1,25(OH)₂D also alter plasma calcium and PTH levels.

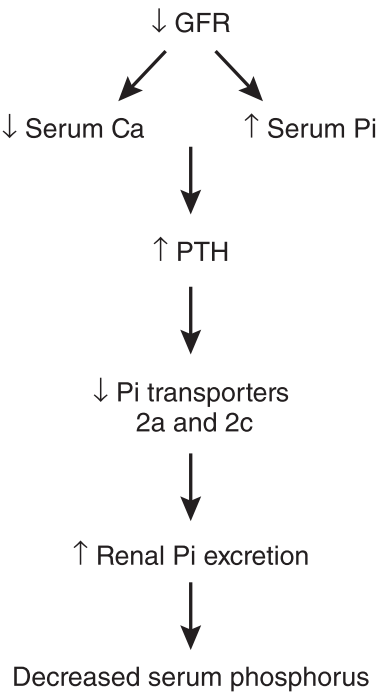


Figure 7. | Schematic of PTH-induced homeostatic mechanisms to correct hyperphosphatemia as GFR falls. As GFR falls, serum phosphorus levels increase, which stimulates release of PTH from the parathyroid glands. This in turn decreases the brush border abundance of Npt2a and Npt2c in the renal proximal tubule, leading to increased urinary excretion of phosphorus. PTH, parathyroid hormone.

Glucocorticoids. Increased glucocorticoid levels lead to decreased proximal tubule synthesis and abundance of Npt2a as well as changes in brush border membrane lipid composition, which is thought to modulate sodium phosphate cotransporter activity (45).

Estrogen. Estrogen causes phosphaturia by decreasing the abundance of Npt2a in the proximal tubule without altering Npt2c levels (46). Estrogen also increases FGF23 synthesis (47).

Thyroid Hormone. Increased levels of thyroid hormone increase phosphate absorption by increasing proximal tubule transcription and expression of Npt2a (48). The Npt2a gene contains a thyroid response element and transcription of Npt2a mRNA is regulated by 3,5,3-triiodothyronine (49).

Dopamine. Dopamine leads to phosphaturia by inducing internalization of Npt2a from the proximal tubule brush border membrane. Dopamine-mediated internalization of Npt2a is dependent on a scaffolding protein (sodium-hydrogen exchanger regulatory factor 1) because dopamine does not induce phosphaturia in sodium-hydrogen exchanger regulatory factor 1 knockout mice (50).

Metabolic Acidosis. Metabolic acidosis stimulates phosphaturia, which helps remove acid from the blood because phosphate serves as a titratable acid (51). By contrast, metabolic alkalosis increases renal phosphate absorption (52). In mice, acidosis increases proximal tubule brush border membrane abundance of Npt2a and Npt2c, suggesting that phosphaturia results from inhibition of sodium phosphate cotransporter activity rather than changes in the levels of these proteins.

Hypertension. An acute increase in BP leads to decreased renal phosphate reabsorption by inducing removal of Npt2a from the proximal tubule brush border membrane microvilli to subapical endosomes (53).

Clinical Consequences of Alterations in Phosphorus Balance

Hypophosphatemia. Hypophosphatemia can be caused by a shift of phosphorus from extracellular fluid into cells, decreased intestinal absorption of phosphate, or increased renal excretion of phosphate (54). Hypophosphatemia is also seen in rare genetic disorders that decrease renal sodium phosphate cotransporter activity or increase PTH or FGF23 levels (55). Hypophosphatemia is generally asymptomatic but serum phosphate levels <1.5 mg/dl can cause anorexia, confusion, rhabdomyolysis, paralysis, and seizures. Respiratory depression in particular is associated with serum levels <1 mg/dl (56). Mild hypophosphatemia is treated with oral supplementation, whereas severe hypophosphatemia requires intravenous repletion (55).

Hyperphosphatemia. As the GFR falls, free serum calcium levels fall and serum phosphorus increases (21). The decrease in free serum calcium and increase in serum phosphorus stimulate the parathyroid glands to produce PTH, which decreases the abundance of Npt2a and Npt2c in the renal proximal tubule, leading to increased Pi excretion in the urine that in turn lowers serum Pi levels (Figure 6). Hyperphosphatemia also stimulates production of FGF23, which decreases levels of Npt2a and Npt2c in the kidney, resulting in increased urinary excretion of phosphate (Figure 7). FGF23 also decreases production of 1,25(OH)₂D, which decreases intestinal Pi absorption, further

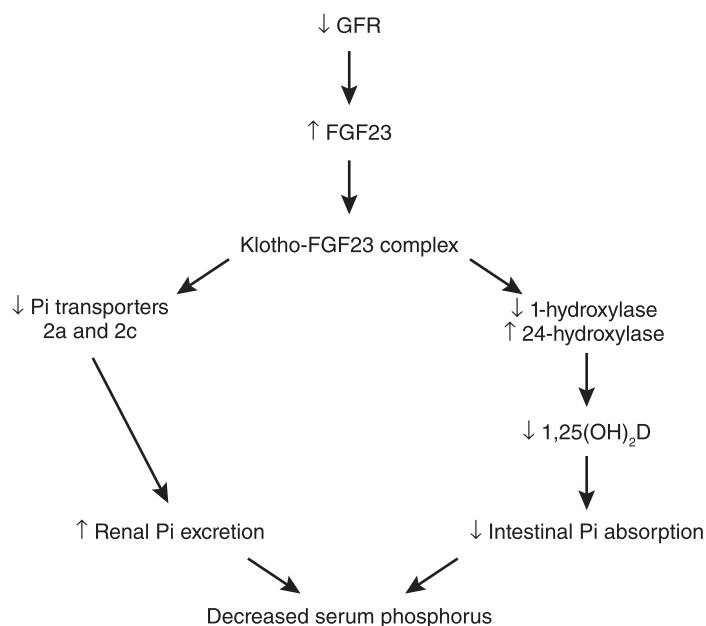


Figure 8. | Schematic of the role of FGF23 in reducing hyperphosphatemia as GFR falls. As GFR falls, serum phosphorus levels increase, leading to increased FGF23 secretion from bone. FGF23 binds Klotho, a required cofactor, and the FGF23/Klotho complex binds and activates FGFR1c, leading to decreased renal tubular expression of Npt2a and Npt2c. In addition, activation of FGFR1c by the FGF23/Klotho complex results in decreased 1α -hydroxylase activity and increased 24α -hydroxylase activity, which leads to decreased levels of $1,25(\text{OH})_2\text{D}$ and decreased Pi absorption from the gut. FGF23, fibroblast growth factor-23; FGFR1c, FGF23 receptor 1c.

decreasing serum Pi levels (21). As GFR continues to fall, however, these compensatory mechanisms fail, leading to hyperphosphatemia.

Acute elevations in serum phosphorus levels caused by oral sodium phosphate bowel purgatives (*e.g.*, used in preparation for colonoscopy) can cause acute phosphate nephropathy (57). Acute phosphate nephropathy is characterized by AKI leading to chronic renal failure and biopsy findings of tubular injury and tubular and interstitial calcium phosphate deposits.

Hyperphosphatemia is strongly associated with increased cardiovascular morbidity and mortality and increases the risk of calciphylaxis (58). Current treatment of hyperphosphatemia involves the use of oral phosphate binders that block phosphorus absorption from food.

Secondary Hyperparathyroidism

As mentioned above, under physiologic circumstances, PTH maintains serum calcium, increases phosphorus excretion, and stimulates production of $1,25(\text{OH})_2\text{D}$. However, as kidney function declines, PTH rises. A decrease in GFR causes an increase in serum phosphorus and FGF23 levels, which, consequently, decreases 1α -hydroxylase activity and $1,25(\text{OH})_2\text{D}$ synthesis. The decrease in serum concentrations of $1,25(\text{OH})_2\text{D}$ decreases serum calcium levels and secondary hyperparathyroidism ensues (16). Although FGF23 is known to suppress PTH in a nonuremic milieu, the parathyroid gland becomes resistant to FGF23 with progressive kidney disease, favoring PTH release and worsening of secondary hyperparathyroidism (59). Hence, the lack of $1,25(\text{OH})_2\text{D}$ and increased FGF23 allow increased PTH production to continue (Figure 9).

The management of secondary hyperparathyroidism in dialysis patients principally involves the administration of some combination of the following: phosphate binders, vitamin D analogs, and calcimimetics. The CaSR is one of the main factors regulating PTH synthesis and secretion; therefore, this target offers the potential to suppress PTH by complementary mechanism to vitamin D analogs. Calcimimetics are agents that allosterically increase the sensitivity of the CaSR in the parathyroid gland to calcium (22).

Magnesium

Regulation of Magnesium Homeostasis

Magnesium is the second most abundant intracellular divalent cation. Magnesium serves several important functions in the human body, including intracellular signaling, serving as a cofactor for protein and DNA synthesis, oxidative phosphorylation, cardiovascular tone, neuromuscular excitability, and bone formation (60–64).

The total body magnesium content in adults is approximately 24 g, 99% of which is intracellular, stored predominantly in bone, muscle, and soft tissue, with only 1% in the extracellular space. Normal total serum magnesium concentration is in the range of 0.7–1.1 mmol/l, 1.4–2.2 mEq/l, or 1.7–2.6 mg/dl. Sixty percent of serum magnesium exists in the ionized, free, physiologically active form, which is important for its physiologic functions. Ten percent of serum magnesium exists complexed to serum anions. Thirty percent of serum magnesium is albumin-bound (60–64).

As detailed below, serum magnesium concentration is regulated by the dynamic balance and interplay between intestinal and renal transport and bone exchange (Figure 1C).

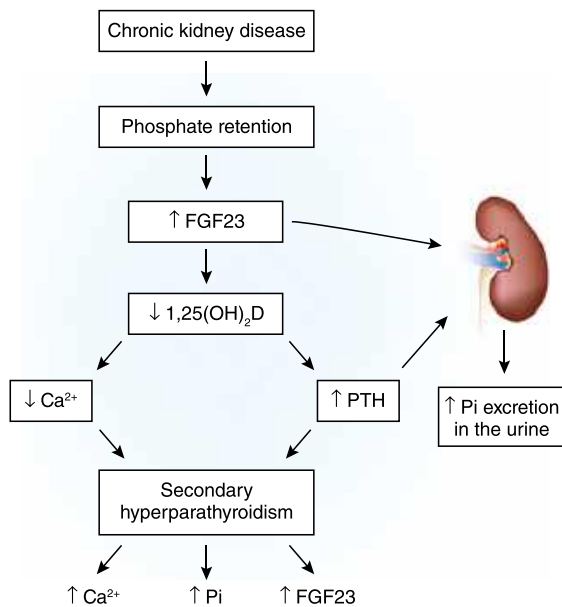


Figure 9. | Pathogenesis of secondary hyperparathyroidism in CKD.

Progressive loss of renal mass impairs renal phosphate excretion, which causes an increase in serum phosphorus. Abnormalities in serum phosphorus homeostasis stimulate FGF23 from bone. Higher serum FGF23 levels in addition to decreased renal mass cause a quantitative decrease in synthesis of 1,25(OH)₂D. High serum FGF23 levels decrease the activity of the 1 α -hydroxylase enzyme. 1,25(OH)₂D deficiency decreases intestinal absorption of calcium, leading to hypocalcemia, which is augmented by the direct effect of hyperphosphatemia. Hypocalcemia and hyperphosphatemia stimulate PTH release and synthesis. The lack of 1,25(OH)₂D, which would ordinarily feed back to inhibit the transcription of prepro-PTH and exert an antiproliferative effect on parathyroid cells, allows the increased PTH production to continue. Current therapeutic methods used to decrease PTH release in CKD include correction of hyperphosphatemia, maintenance of normal serum calcium levels, administration of 1,25(OH)₂D analogs orally or intravenously, and administration of a CaSR agonist (e.g., cinacalcet).

Intestinal Magnesium Absorption

Typical magnesium ingestion is approximately 300 mg/d (65). Intestinal absorption can range from 25% when eating magnesium-rich diets to 75% when eating magnesium-depleted diets. Approximately 120 mg of magnesium is absorbed and 20 mg is lost in gastrointestinal secretions, amounting to a net daily intake of 100 mg/d. Intestinal magnesium absorption occurs *via* a saturable transcellular pathway and a nonsaturable paracellular passive pathway (65,66) (Figure 2C). The majority of magnesium is absorbed by the small intestine and, to a lesser extent, by the colon. Transcellular magnesium absorption is permitted by the transient receptor potential melastatin (TRPM) cationic channels TRPM6 and TRPM7. Mutations in TRPM6 result in hypomagnesemia with secondary hypocalcemia (67,68). TRPM7 also plays an important role in intestinal magnesium absorption because heterozygote TRPM7-deficient mice develop hypomagnesemia due to decreased intestinal magnesium absorption, whereas renal magnesium excretion is low to compensate for the decreased intestinal magnesium absorption (69). When dietary magnesium intake is

normal, transcellular transport mediates 30% of intestinal magnesium absorption. This fraction increases when dietary magnesium intake is lower (70–72). When dietary magnesium is higher, then the majority of intestinal magnesium absorption occurs *via* the paracellular pathway, which is regulated by proteins comprising the tight junction, including claudins, occludin, and zona-occludens-1 (73–76). Tight junction assembly and function can be modulated by a number of signaling molecules that alter the phosphorylation state of the tight junctional proteins and the ionic permeability of the paracellular pathway. Paracellular transport depends on the transepithelial electrical voltage, which is approximately 5 mV lumen-positive with respect to blood. In addition, luminal magnesium concentrations range between 1.0 and 5.0 mmol/l compared with serum magnesium concentrations of between 0.70 and 1.10 mmol/l, which also provides a transepithelial chemical concentration gradient favoring absorption.

Proton pump inhibitors, especially after long-term use, have been associated with hypomagnesemia. Impaired intestinal magnesium absorption, rather than renal absorption, seems to mediate the effect of proton pump inhibitors. Potential mediators include increased intestinal magnesium secretion, decreased active transcellular magnesium absorption due to decreased TRPM6 activity secondary to decreased intestinal acidity, or decreased paracellular magnesium absorption (77,78).

Renal Regulation of Magnesium

Assuming a normal GFR, the kidney filters approximately 2000–2400 mg of magnesium per day. This takes into account the fact that only 70% of total serum magnesium (30% is protein-bound) is available for glomerular filtration. Under normal conditions, 96% of filtered magnesium is reabsorbed in the renal tubules by several coordinated transport processes and magnesium transporters detailed below (9,12,79–84).

Proximal Tubule. As shown in Figure 3C, 10%–30% of the filtered magnesium is absorbed in the proximal tubule. Although the exact mechanisms are not known, magnesium is believed to be absorbed *via* a paracellular pathway aided by a chemical gradient generated by Na gradient-driven water transport that increases intraluminal magnesium as well as lumen-positive potential.

Thick Ascending Limb. A paracellular pathway in the thick ascending limb absorbs 40%–70% of filtered magnesium, mostly enhanced by lumen-positive transepithelial voltage, in which claudin-16 and claudin-19 play an important role. The NKCC2 cotransporter mediates apical absorption of Na, K, and Cl. The apical ROMK mediates apical recycling of K back to the tubular lumen and generation of lumen-positive voltage. The Cl channel CIC-Kb mediates Cl exit through the basolateral membrane. Na, K-ATPase also mediates Na exit through the basolateral membrane and generates the Na gradient for Na absorption. The tight junction proteins claudin-16 and claudin-19 play a prominent role in magnesium absorption. The CaSR has also been determined to regulate magnesium transport in this segment: upon stimulation, magnesium transport is decreased. Basolateral receptor activation inhibits apical K channels and possibly Na-2Cl-K cotransport in the rat thick ascending limb (85,86). This inhibition would be expected to

Table 3. Factors that alter renal regulation of magnesium

Increase Magnesium Absorption	Decrease Magnesium Absorption
Dietary magnesium restriction	Hypermagnesemia
Parathyroid hormone	Metabolic acidosis
Glucagon	Hypercalcemia
Calcitonin	Phosphate depletion
Vasopressin	Potassium depletion
Aldosterone	Diuretics (loop and thiazide)
Insulin	Antibiotics (aminoglycosides)
Amiloride	Antifungals (amphotericin B)
Metabolic alkalosis	Antivirals (foscarnet)
EGF	Chemotherapy agents (cisplatin)
	Immunosuppressants (tacrolimus, cyclosporine, rapamycin)
	EGF receptor antagonists
	FHHNC caused by mutations in claudin-16 and claudin-19
	HSH caused by mutations in TRPM6
	Bartter's syndrome caused by mutations in NKCC2 (type I), ROMK (type II), CLC-Kb (type III), or CaSR (type V)
	Dominant hypomagnesemia caused by mutations of the FXYP2 gene, HNF1B, or CNNM2
	Isolated dominant hypomagnesemia caused by mutations of Kv1.1
	Isolated recessive hypomagnesemia caused by mutations of pro-EGF
	Gitelman's syndrome caused by mutations of NCC
	EAST/SeSAME caused by mutations in Kir4.1
FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HSH, hypomagnesemia with secondary hypocalcemia; TRPM6, transient receptor potential melastatin 6; NKCC2, Na ⁺ -K ⁺ -2Cl ⁻ cotransporter; ROMK, renal outer medullary potassium K ⁺ ; CLC-Kb, Cl channel Kb; CaSR, calcium-sensing receptor; FXYP2, sodium/potassium-transporting ATPase gamma chain (a protein that in humans is encoded by the FXYP2 gene); HNF1B, hepatocyte nuclear factor 1 beta; CNNM2, cyclin M2; NCC, thiazide-sensitive Na-Cl Cotransporter; EAST (epilepsy, ataxia, sensorineural deafness, and tubulopathy)/SeSAME (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance).	

diminish transepithelial voltage and, in turn, passive transport of magnesium within the cortical thick ascending limb.

Bartter's syndrome is caused by mutations in NKCC2, or ROMK, or CLC-Kb, or Barttin (an essential β -subunit for CLC-Ka and CLC-Kb chloride channels), and/or CaSR.

However, hypomagnesemia is not present in all patients with Bartter's syndrome.

Mutations in CLDN16 or CLDN19 encoding the tight junction proteins claudin-16 and claudin-19 result in increased urinary magnesium excretion, hypomagnesemia, increased urinary calcium excretion, and nephrocalcinosis (autosomal recessive familial hypomagnesemia with hypercalciuria and nephrocalcinosis).

By contrast, a recent study indicates a different phenotype for mutations in claudin-10 (87). Claudin-10 determines paracellular sodium permeability and its loss leads to hypermagnesemia and nephrocalcinosis. In isolated perfused thick ascending limb tubules of claudin-10-deficient mice, paracellular permeability of sodium is decreased and the relative permeability of calcium and magnesium is increased. Moreover, furosemide-inhibitable transepithelial voltage is increased, leading to a shift from paracellular sodium transport to paracellular hyperabsorption of calcium and magnesium. These data identify claudin-10 as a key factor in control of cation selectivity and transport in the thick ascending limb, and deficiency in this pathway as a cause of nephrocalcinosis.

Loop diuretics inhibit chloride (Cl⁻) absorption by NKCC2 and also decrease basolateral Cl⁻ efflux. This results in loss of lumen-positive potential, thereby decreasing the driving force for paracellular magnesium reabsorption *via* claudin-16 and claudin-19.

Distal Convoluted Tubule. The remaining 5%–10% of magnesium is reabsorbed in the distal convoluted tubule mainly by active transcellular transport mediated by TRPM6. The apical K channel Kv1.1 potentiates TRPM6-mediated magnesium absorption by establishing favorable luminal potential. EGF increases magnesium transport through TRPM6. Inhibitors of EGF receptors such as cetuximab or panitumumab, utilized for treatment of EGF responsive cancers, are able to induce hypomagnesemia by interfering with magnesium transport in the kidney (88).

Basolateral K channel Kir4.1 and the γ -subunit of Na, K-ATPase also increase magnesium reabsorption by generating a sodium gradient, making it possible for the thiazide-sensitive NCC (thiazide-sensitive Na-Cl Cotransporter) to facilitate sodium transport from the apical lumen to the cytosol.

The absorbed magnesium is then extruded *via* a recently identified magnesium/sodium exchanger SLC41A1 family (89–92) across the basolateral membrane (93). Interestingly, mutations in SLC41A1 result in a nephronophthisis-like phenotype (93). In addition, CNNM2 (cyclin M2) has also been identified as a gene involved in renal Mg²⁺ handling in patients of two unrelated families with unexplained dominant hypomagnesemia. In the kidney, CNNM2 was predominantly found along the basolateral membrane of distal tubular segments involved in Mg²⁺ reabsorption (94).

In addition to TRPM6, the importance of these transporters in the thick ascending limb and distal convoluted tubule as well as the tight junction proteins in the thick ascending limb to regulation of magnesium reabsorption is reflected by the fact that mutations of these proteins result in various forms of genetic syndromes of hypomagnesemia (Table 3).

Mutations in PCBD1 (pterin-4 α -carbinolamine dehydratase/dimerization cofactor of hepatocyte NF1 homeobox A) were recently shown to cause hypomagnesemia secondary to renal magnesium wasting (95). PCBD1 is a dimerization

cofactor for the transcription factor HNF1B. PCDB1 binds HNF1B to costimulate the FXD2 (sodium/potassium-transporting ATPase gamma chain [a protein that in humans is encoded by the FXD2 gene]) promoter, which increases renal tubular magnesium reabsorption in the distal convoluted tubule.

Bone. At least 50% of the total body magnesium content resides in bone as hydroxyapatite crystals (96). Dietary magnesium restriction causes decreased bone magnesium content (97). Hypomagnesemia in rodents induces osteopenia with accelerated bone turnover, decreased bone volume, and decreased bone strength (98–101). Although the bone magnesium stores are dynamic, the transporters that mediate magnesium flux in and out of bone have not yet been determined.

Clinical Consequences of Alterations in Magnesium Balance

Hypomagnesemia. Hypomagnesemia is usually defined as serum magnesium <0.7 mmol/L, 1.4 mEq/L, or 1.7 mg/dL. Biochemical hypomagnesemia is common, with a prevalence of up to 15% in the general population and up to 65% in patients in the intensive care units (60–64,102–109). The causes of hypomagnesemia are listed in Table 3.

Hypomagnesemia can be secondary to impaired intestinal magnesium absorption or increased urinary magnesium excretion secondary to various hormones or drugs that inhibit magnesium reabsorption. At the clinical level, the assessment of magnesium stores and cause of magnesium deficiency continues to be a real challenge. Simultaneous measurements of serum and urine magnesium may help differentiate the cause of hypomagnesemia.

Although proton pump inhibitors most likely cause impaired intestinal magnesium absorption, most of the other drugs associated with hypomagnesemia impair renal tubular magnesium reabsorption by direct or indirect inhibition of magnesium reabsorption in the thick ascending limb or the distal convoluted tubule (102,110,111).

Hypomagnesemia is associated with hypokalemia, which is mediated by stimulation of the ROMK channel resulting in increased potassium excretion. Hypomagnesemia is also associated with hypocalcemia secondary to impaired PTH release and PTH resistance. Clinical manifestations of hypomagnesemia include weakness and fatigue, muscle cramps, tetany, numbness, seizures, and arrhythmias.

Hypermagnesemia. Hypermagnesemia is caused by ingestion and increased intestinal absorption of Epsom salts and magnesium-containing cathartics, antacids, laxative abuse, and enemas. In addition, overzealous intravenous or intramuscular injection of magnesium for treatment of preeclampsia can also result in hypermagnesemia.

Hypermagnesemia is associated with nausea, vomiting, neurologic impairment, hypotension, and electrocardiography changes including prolonged QRS, PR, and QT intervals. At higher levels due to intoxication, complete heart block, respiratory paralysis, coma, and shock can occur.

Maintenance of normal serum levels of calcium, phosphorus, and magnesium depends on a complex interplay between absorption from the gut, exchange from bone stores, and renal regulation. Renal reabsorption of calcium, phosphorus, and magnesium occurs in several different parts of the nephron and involves a number of channels, transporters, and paracellular pathways, some of which

remain to be defined. The importance of the kidney in maintaining normal calcium, phosphorus, and magnesium homeostasis can be seen in renal failure in which abnormalities of calcium, phosphorus, and magnesium levels are very common clinical findings.

Disclosures

M.L. has received grant support (unrelated to this publication) from the National Institutes of Health, US Department of Veterans Affairs, Intercept, Daiichi Sankyo, Abbott, Genzyme, and Novartis.

References

- Kumar R: Calcium metabolism. In: *The Principles and Practice of Nephrology*, edited by Jacobson HR, Striker GE, Klahr S, 2nd Ed., St. Louis, MO, Mosby-Year Book, 1995, pp 964–971
- Hebert SC, Brown EM: The scent of an ion: Calcium-sensing and its roles in health and disease. *Curr Opin Nephrol Hypertens* 5: 45–53, 1996
- Johnson JA, Kumar R: Renal and intestinal calcium transport: Roles of vitamin D and vitamin D-dependent calcium binding proteins. *Semin Nephrol* 14: 119–128, 1994
- Kumar R: Calcium transport in epithelial cells of the intestine and kidney. *J Cell Biochem* 57: 392–398, 1995
- Keller J, Schinke T: The role of the gastrointestinal tract in calcium homeostasis and bone remodeling. *Osteoporos Int* 24: 2737–2748, 2013
- Friedman PA, Gesek FA: Cellular calcium transport in renal epithelia: Measurement, mechanisms, and regulation. *Physiol Rev* 75: 429–471, 1995
- Felsenfeld A, Rodriguez M, Levine B: New insights in regulation of calcium homeostasis. *Curr Opin Nephrol Hypertens* 22: 371–376, 2013
- Riccardi D, Brown EM: Physiology and pathophysiology of the calcium-sensing receptor in the kidney. *Am J Physiol Renal Physiol* 298: F485–F499, 2010
- Ferrè S, Hoenderop JG, Bindels RJ: Sensing mechanisms involved in Ca²⁺ and Mg²⁺ homeostasis. *Kidney Int* 82: 1157–1166, 2012
- Houillier P: Calcium-sensing in the kidney. *Curr Opin Nephrol Hypertens* 22: 566–571, 2013
- Loupy A, Ramakrishnan SK, Wootla B, Chambrey R, de la Faille R, Bourgeois S, Bruneval P, Mandet C, Christensen EI, Faure H, Cheval L, Laghmani K, Collet C, Eladari D, Dodd RH, Ruat M, Houillier P: PTH-independent regulation of blood calcium concentration by the calcium-sensing receptor. *J Clin Invest* 122: 3355–3367, 2012
- Toka HR, Al-Romaih K, Koshy JM, DiBartolo S 3rd, Kos CH, Quinn SJ, Curhan GC, Mount DB, Brown EM, Pollak MR: Deficiency of the calcium-sensing receptor in the kidney causes parathyroid hormone-independent hypocalciuria. *J Am Soc Nephrol* 23: 1879–1890, 2012
- Lambers TT, Bindels RJ, Hoenderop JG: Coordinated control of renal Ca²⁺ handling. *Kidney Int* 69: 650–654, 2006
- Hoenderop JG, Nilius B, Bindels RJ: Molecular mechanism of active Ca²⁺ reabsorption in the distal nephron. *Annu Rev Physiol* 64: 529–549, 2002
- Mensenkamp AR, Hoenderop JG, Bindels RJ: Recent advances in renal tubular calcium reabsorption. *Curr Opin Nephrol Hypertens* 15: 524–529, 2006
- Goodman WG, Quarles LD: Development and progression of secondary hyperparathyroidism in chronic kidney disease: Lessons from molecular genetics. *Kidney Int* 74: 276–288, 2008
- Dusso AS, Brown AJ, Slatopolsky E: Vitamin D. *Am J Physiol Renal Physiol* 289: F8–F28, 2005
- Holick MF: Vitamin D deficiency. *N Engl J Med* 357: 266–281, 2007
- Drüeke TB, Lacour B: Disorders of calcium, phosphate, and magnesium metabolism. In: *Comprehensive Clinical Nephrology*, edited by Feehally J, Floege J, Johnson RJ, 3rd Ed., Philadelphia, Mosby/Elsevier, 2007, pp 123–140
- Biber J, Hernando N, Forster I: Phosphate transporters and their function. *Annu Rev Physiol* 75: 535–550, 2013

21. Marks J, Debnam ES, Unwin RJ: Phosphate homeostasis and the renal-gastrointestinal axis. *Am J Physiol Renal Physiol* 299: F285–F296, 2010
22. Blaine J, Weinman EJ, Cunningham R: The regulation of renal phosphate transport. *Adv Chronic Kidney Dis* 18: 77–84, 2011
23. Forster IC, Hernando N, Biber J, Murer H: Proximal tubular handling of phosphate: A molecular perspective. *Kidney Int* 70: 1548–1559, 2006
24. Azzarolo AM, Ritchie G, Quamme GA: Some characteristics of sodium-independent phosphate transport across renal basolateral membranes. *Biochim Biophys Acta* 1064: 229–234, 1991
25. Murer H, Forster I, Biber J: The sodium phosphate cotransporter family SLC34. *Pflugers Arch* 447: 763–767, 2004
26. Magagnin S, Werner A, Markovich D, Sorribas V, Stange G, Biber J, Murer H: Expression cloning of human and rat renal cortex Na/Pi cotransport. *Proc Natl Acad Sci U S A* 90: 5979–5983, 1993
27. Segawa H, Kaneko I, Takahashi A, Kuwahata M, Ito M, Ohkido I, Tatsumi S, Miyamoto K: Growth-related renal type II Na/Pi cotransporter. *J Biol Chem* 277: 19665–19672, 2002
28. Villa-Belostta R, Ravera S, Sorribas V, Stange G, Levi M, Murer H, Biber J, Forster IC: The Na⁺-Pi cotransporter PiT-2 (SLC20A2) is expressed in the apical membrane of rat renal proximal tubules and regulated by dietary Pi. *Am J Physiol Renal Physiol* 296: F691–F699, 2009
29. Segawa H, Onitsuka A, Furutani J, Kaneko I, Aranami F, Matsumoto N, Tomoe Y, Kuwahata M, Ito M, Matsumoto M, Li M, Amizuka N, Miyamoto K: Npt2a and Npt2c in mice play distinct and synergistic roles in inorganic phosphate metabolism and skeletal development. *Am J Physiol Renal Physiol* 297: F671–F678, 2009
30. Beck L, Karaplis AC, Amizuka N, Hewson AS, Ozawa H, Tenenhouse HS: Targeted inactivation of Npt2 in mice leads to severe renal phosphate wasting, hypercalciuria, and skeletal abnormalities. *Proc Natl Acad Sci U S A* 95: 5372–5377, 1998
31. Tenenhouse HS, Martel J, Gauthier C, Segawa H, Miyamoto K: Differential effects of Npt2a gene ablation and X-linked Hyp mutation on renal expression of Npt2c. *Am J Physiol Renal Physiol* 285: F1271–F1278, 2003
32. Bacconi A, Virkki LV, Biber J, Murer H, Forster IC: Renouncing electroneutrality is not free of charge: Switching on electrogenic in a Na⁺-coupled phosphate cotransporter. *Proc Natl Acad Sci U S A* 102: 12606–12611, 2005
33. Virkki LV, Forster IC, Biber J, Murer H: Substrate interactions in the human type IIa sodium-phosphate cotransporter (NaPi-IIa). *Am J Physiol Renal Physiol* 288: F969–F981, 2005
34. Ravera S, Virkki LV, Murer H, Forster IC: Deciphering PiT transport kinetics and substrate specificity using electrophysiology and flux measurements. *Am J Physiol Cell Physiol* 293: C606–C620, 2007
35. Virkki LV, Biber J, Murer H, Forster IC: Phosphate transporters: A tale of two solute carrier families. *Am J Physiol Renal Physiol* 293: F643–F654, 2007
36. Breusegem SY, Takahashi H, Giral-Arnal H, Wang X, Jiang T, Verlander JW, Wilson P, Miyazaki-Anzai S, Sutherland E, Caldas Y, Blaine JT, Segawa H, Miyamoto K, Barry NP, Levi M: Differential regulation of the renal sodium-phosphate cotransporters NaPi-IIa, NaPi-IIc, and PiT-2 in dietary potassium deficiency. *Am J Physiol Renal Physiol* 297: F350–F361, 2009
37. Déliot N, Hernando N, Horst-Liu Z, Gisler SM, Capuano P, Wagner CA, Bacic D, O'Brien S, Biber J, Murer H: Parathyroid hormone treatment induces dissociation of type IIa Na⁺-(Pi) cotransporter-Na⁺/H⁺ exchanger regulatory factor-1 complexes. *Am J Physiol Cell Physiol* 289: C159–C167, 2005
38. Millar AL, Jackson NA, Dalton H, Jennings KR, Levi M, Wharen B, Dimmock NJ: Rapid analysis of epitope-paratope interactions between HIV-1 and a 17-amino-acid neutralizing microantibody by electrospray ionization mass spectrometry. *Eur J Biochem* 258: 164–169, 1998
39. Lederer ED, Khundmiri SJ, Weinman EJ: Role of NHERF-1 in regulation of the activity of Na-K ATPase and sodium-phosphate co-transport in epithelial cells. *J Am Soc Nephrol* 14: 1711–1719, 2003
40. Mahon MJ, Cole JA, Lederer ED, Segre GV: Na⁺/H⁺ exchanger-regulatory factor 1 mediates inhibition of phosphate transport by parathyroid hormone and second messengers by acting at multiple sites in opossum kidney cells. *Mol Endocrinol* 17: 2355–2364, 2003
41. Blaine J, Okamura K, Giral H, Breusegem S, Caldas Y, Millard A, Barry N, Levi M: PTH-induced internalization of apical membrane NaPi2a: Role of actin and myosin VI. *Am J Physiol Cell Physiol* 297: C1339–C1346, 2009
42. Berndt T, Kumar R: Phosphatonins and the regulation of phosphate homeostasis. *Annu Rev Physiol* 69: 341–359, 2007
43. Hasegawa H, Nagano N, Urakawa I, Yamazaki Y, Iijima K, Fujita T, Yamashita T, Fukumoto S, Shimada T: Direct evidence for a causative role of FGF23 in the abnormal renal phosphate handling and vitamin D metabolism in rats with early-stage chronic kidney disease. *Kidney Int* 78: 975–980, 2010
44. Kurnik BR, Hruska KA: Mechanism of stimulation of renal phosphate transport by 1,25-dihydroxycholecalciferol. *Biochim Biophys Acta* 817: 42–50, 1985
45. Levi M, Shayman JA, Abe A, Gross SK, McCluer RH, Biber J, Murer H, Lötscher M, Cronin RE: Dexamethasone modulates rat renal brush border membrane phosphate transporter mRNA and protein abundance and glycosphingolipid composition. *J Clin Invest* 96: 207–216, 1995
46. Farouqi S, Levi M, Soleimani M, Amlal H: Estrogen down-regulates the proximal tubule type IIa sodium phosphate cotransporter causing phosphate wasting and hypophosphatemia. *Kidney Int* 73: 1141–1150, 2008
47. Cannata-Andía JB, Carrillo-López N, Naves-Díaz M: Estrogens and bone disease in chronic kidney disease: Role of FGF23. *Curr Opin Nephrol Hypertens* 19: 354–358, 2010
48. Alcalde AI, Sarasa M, Raldúa D, Aramayona J, Morales R, Biber J, Murer H, Levi M, Sorribas V: Role of thyroid hormone in regulation of renal phosphate transport in young and aged rats. *Endocrinology* 140: 1544–1551, 1999
49. Ishiguro M, Yamamoto H, Masuda M, Kozai M, Takei Y, Tanaka S, Sato T, Segawa H, Taketani Y, Arai H, Miyamoto K, Takeda E: Thyroid hormones regulate phosphate homeostasis through transcriptional control of the renal type IIa sodium-dependent phosphate co-transporter (Npt2a) gene. *Biochem J* 427: 161–169, 2010
50. Weinman EJ, Biswas R, Steplock D, Douglass TS, Cunningham R, Shenolikar S: Sodium-hydrogen exchanger regulatory factor 1 (NHERF-1) transduces signals that mediate dopamine inhibition of sodium-phosphate co-transport in mouse kidney. *J Biol Chem* 285: 13454–13460, 2010
51. Nowik M, Picard N, Stange G, Capuano P, Tenenhouse HS, Biber J, Murer H, Wagner CA: Renal phosphaturia during metabolic acidosis revisited: Molecular mechanisms for decreased renal phosphate reabsorption. *Pflugers Arch* 457: 539–549, 2008
52. Biber J, Hernando N, Forster I, Murer H: Regulation of phosphate transport in proximal tubules. *Pflugers Arch* 458: 39–52, 2009
53. Magyar CE, Zhang Y, Holstein-Rathlou NH, McDonough AA: Proximal tubule Na transporter responses are the same during acute and chronic hypertension. *Am J Physiol Renal Physiol* 279: F358–F369, 2000
54. Felsenfeld AJ, Levine BS: Approach to treatment of hypophosphatemia. *Am J Kidney Dis* 60: 655–661, 2012
55. Carpenter TO: The expanding family of hypophosphatemic syndromes. *J Bone Miner Metab* 30: 1–9, 2012
56. Gravelyn TR, Brophy N, Siegert C, Peters-Golden M: Hypophosphatemia-associated respiratory muscle weakness in a general inpatient population. *Am J Med* 84: 870–876, 1988
57. Markowitz GS, Perazella MA: Acute phosphate nephropathy. *Kidney Int* 76: 1027–1034, 2009
58. Kendrick J, Kestenbaum B, Chonchol M: Phosphate and cardiovascular disease. *Adv Chronic Kidney Dis* 18: 113–119, 2011
59. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, Sirkis R, Naveh-Manly T, Silver J: The parathyroid is a target organ for FGF23 in rats. *J Clin Invest* 117: 4003–4008, 2007
60. Whang R, Hampton EM, Whang DD: Magnesium homeostasis and clinical disorders of magnesium deficiency. *Ann Pharmacother* 28: 220–226, 1994

61. Konrad M, Schlingmann KP, Gudermann T: Insights into the molecular nature of magnesium homeostasis. *Am J Physiol Renal Physiol* 286: F599–F605, 2004
62. Topf JM, Murray PT: Hypomagnesemia and hypermagnesemia. *Rev Endocr Metab Disord* 4: 195–206, 2003
63. Ayuk J, Gittoes NJ: How should hypomagnesaemia be investigated and treated? *Clin Endocrinol (Oxf)* 75: 743–746, 2011
64. Agus ZS: Hypomagnesemia. *J Am Soc Nephrol* 10: 1616–1622, 1999
65. Quamme GA: Recent developments in intestinal magnesium absorption. *Curr Opin Gastroenterol* 24: 230–235, 2008
66. Fine KD, Santa Ana CA, Porter JL, Fordtran JS: Intestinal absorption of magnesium from food and supplements. *J Clin Invest* 88: 396–402, 1991
67. Schlingmann KP, Weber S, Peters M, Niemann Nejsum L, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M: Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet* 31: 166–170, 2002
68. Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC: Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nat Genet* 31: 171–174, 2002
69. Ryazanova LV, Rondon LJ, Zierler S, Hu Z, Galli J, Yamaguchi TP, Mazur A, Fleig A, Ryazanov AG: TRPM7 is essential for Mg (2+) homeostasis in mammals. *Nat Commun* 1: 109, 2010
70. Milla PJ, Aggett PJ, Wolff OH, Harries JT: Studies in primary hypomagnesaemia: Evidence for defective carrier-mediated small intestinal transport of magnesium. *Gut* 20: 1028–1033, 1979
71. Karbach U: Cellular-mediated and diffusive magnesium transport across the descending colon of the rat. *Gastroenterology* 96: 1282–1289, 1989
72. Jüttner R, Ebel H: Characterization of Mg2+ transport in brush border membrane vesicles of rabbit ileum studied with magfura-2. *Biochim Biophys Acta* 1370: 51–63, 1998
73. Anderson JM, Van Itallie CM: Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol* 1: a002584, 2009
74. Shen L, Weber CR, Raleigh DR, Yu D, Turner JR: Tight junction pore and leak pathways: A dynamic duo. *Annu Rev Physiol* 73: 283–309, 2011
75. Amasheh S, Fromm M, Günzel D: Claudins of intestine and nephron - A correlation of molecular tight junction structure and barrier function. *Acta Physiol (Oxf)* 201: 133–140, 2011
76. Günzel D, Yu AS: Claudins and the modulation of tight junction permeability. *Physiol Rev* 93: 525–569, 2013
77. Lameris AL, Hess MW, van Kruijlsbergen I, Hoenderop JG, Bindels RJ: Omeprazole enhances the colonic expression of the Mg(2+) transporter TRPM6. *Pflügers Arch* 465: 1613–1620, 2013
78. Hess MW, Hoenderop JG, Bindels RJ, Drenth JP: Systematic review: Hypomagnesaemia induced by proton pump inhibition. *Aliment Pharmacol Ther* 36: 405–413, 2012
79. Bindels RJ: 2009 Homer W. Smith Award: Minerals in motion: From new ion transporters to new concepts. *J Am Soc Nephrol* 21: 1263–1269, 2010
80. Quamme GA: Molecular identification of ancient and modern mammalian magnesium transporters. *Am J Physiol Cell Physiol* 298: C407–C429, 2010
81. Ferrè S, Hoenderop JG, Bindels RJ: Insight into renal Mg2+ transporters. *Curr Opin Nephrol Hypertens* 20: 169–176, 2011
82. Tyler Miller R: Control of renal calcium, phosphate, electrolyte, and water excretion by the calcium-sensing receptor. *Best Pract Res Clin Endocrinol Metab* 27: 345–358, 2013
83. Haisch L, Almeida JR, Abreu da Silva PR, Schlingmann KP, Konrad M: The role of tight junctions in paracellular ion transport in the renal tubule: Lessons learned from a rare inherited tubular disorder. *Am J Kidney Dis* 57: 320–330, 2011
84. Haisch L, Konrad M: Impaired paracellular ion transport in the loop of Henle causes familial hypomagnesemia with hypercalciuria and nephrocalcinosis. *Ann N Y Acad Sci* 1258: 177–184, 2012
85. Hebert SC: Extracellular calcium-sensing receptor: Implications for calcium and magnesium handling in the kidney. *Kidney Int* 50: 2129–2139, 1996
86. Wang WH, Lu M, Hebert SC: Cytochrome P-450 metabolites mediate extracellular Ca(2+)-induced inhibition of apical K+ channels in the TAL. *Am J Physiol* 271: C103–C111, 1996
87. Breiderhoff T, Himmerkus N, Stuiver M, Mutig K, Will C, Meij IC, Bachmann S, Bleich M, Willnow TE, Müller D: Deletion of claudin-10 (Cldn10) in the thick ascending limb impairs paracellular sodium permeability and leads to hypermagnesemia and nephrocalcinosis. *Proc Natl Acad Sci U S A* 109: 14241–14246, 2012
88. Petrelli F, Borgonovo K, Cabiddu M, Ghilardi M, Barni S: Risk of anti-EGFR monoclonal antibody-related hypomagnesemia: Systematic review and pooled analysis of randomized studies. *Expert Opin Drug Saf* 11[Suppl 1]: S9–S19, 2012
89. Goytain A, Quamme GA: Functional characterization of human SLC41A1, a Mg2+ transporter with similarity to prokaryotic MgtE Mg2+ transporters. *Physiol Genomics* 21: 337–342, 2005
90. Kolisek M, Launay P, Beck A, Sponder G, Serafini N, Brenkus M, Froschauer EM, Martens H, Fleig A, Schweigel M: SLC41A1 is a novel mammalian Mg2+ carrier. *J Biol Chem* 283: 16235–16247, 2008
91. Mandt T, Song Y, Scharenberg AM, Sahni J: SLC41A1 Mg(2+) transport is regulated via Mg(2+)-dependent endosomal recycling through its N-terminal cytoplasmic domain. *Biochem J* 439: 129–139, 2011
92. Kolisek M, Nestler A, Vormann J, Schweigel-Röntgen M: Human gene SLC41A1 encodes for the Na+/Mg+ exchanger. *Am J Physiol Cell Physiol* 302: C318–C326, 2012
93. Hurd TW, Otto EA, Mishima E, Gee HY, Inoue H, Inazu M, Yamada H, Halbritter J, Seki G, Konishi M, Zhou W, Yamane T, Murakami S, Caridi G, Ghiggeri G, Abe T, Hildebrandt F: Mutation of the Mg2+ transporter SLC41A1 results in a nephronophthisis-like phenotype. *J Am Soc Nephrol* 24: 967–977, 2013
94. Stuiver M, Lainez S, Will C, Terry S, Günzel D, Debaix H, Sommer K, Kopplin K, Thumfart J, Kampik NB, Querfeld U, Willnow TE, Némec V, Wagner CA, Hoenderop JG, Devuyst O, Knoers NV, Bindels RJ, Meij IC, Müller D: CNNM2, encoding a basolateral protein required for renal Mg2+ handling, is mutated in dominant hypomagnesemia. *Am J Hum Genet* 88: 333–343, 2011
95. Ferrè S, de Baaij JH, Ferreira P, Germann R, de Klerk JB, Lavrijsen M, van Zeeland F, Venselaar H, Kluijtmans LA, Hoenderop JG, Bindels RJ: Mutations in PCBD1 cause hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol* 25: 574–586, 2014
96. Elin RJ: Assessment of magnesium status. *Clin Chem* 33: 1965–1970, 1987
97. Alfrey AC, Miller NL, Trow R: Effect of age and magnesium depletion on bone magnesium pools in rats. *J Clin Invest* 54: 1074–1081, 1974
98. Lai CC, Singer L, Armstrong WD: Bone composition and phosphatase activity in magnesium deficiency in rats. *J Bone Joint Surg Am* 57: 516–522, 1975
99. Burnell JM, Liu C, Miller AG, Teubner E: Effects of dietary alteration of bicarbonate and magnesium on rat bone. *Am J Physiol* 250: F302–F307, 1986
100. Boskey AL, Rimnac CM, Bansal M, Federman M, Lian J, Boyan BD: Effect of short-term hypomagnesemia on the chemical and mechanical properties of rat bone. *J Orthop Res* 10: 774–783, 1992
101. Kenney MA, McCoy H, Williams L: Effects of magnesium deficiency on strength, mass, and composition of rat femur. *Calcif Tissue Int* 54: 44–49, 1994
102. Lameris AL, Monnens LA, Bindels RJ, Hoenderop JG: Drug-induced alterations in Mg2+ homeostasis. *Clin Sci (Lond)* 123: 1–14, 2012
103. Dimke H, Monnens L, Hoenderop JG, Bindels RJ: Evaluation of hypomagnesemia: Lessons from disorders of tubular transport. *Am J Kidney Dis* 62: 377–383, 2013
104. Whang R, Ryder KW: Frequency of hypomagnesemia and hypermagnesemia. Requested vs routine. *JAMA* 263: 3063–3064, 1990

105. Thienpont LM, Dewitte K, Stöckl D: Serum complexed magnesium—A cautionary note on its estimation and its relevance for standardizing serum ionized magnesium. *Clin Chem* 45: 154–155, 1999
106. Sanders GT, Huijgen HJ, Sanders R: Magnesium in disease: A review with special emphasis on the serum ionized magnesium. *Clin Chem Lab Med* 37: 1011–1033, 1999
107. Huijgen HJ, Soesan M, Sanders R, Mairuhu WM, Kesecioglu J, Sanders GT: Magnesium levels in critically ill patients. What should we measure? *Am J Clin Pathol* 114: 688–695, 2000
108. Kùlpmann WR, Gerlach M: Relationship between ionized and total magnesium in serum. *Scand J Clin Lab Invest Suppl* 224: 251–258, 1996
109. Dewitte K, Dhondt A, Giri M, Stöckl D, Rottiers R, Lameire N, Thienpont LM: Differences in serum ionized and total magnesium values during chronic renal failure between nondiabetic and diabetic patients: A cross-sectional study. *Diabetes Care* 27: 2503–2505, 2004
110. van Angelen AA, Glaudemans B, van der Kemp AW, Hoenderop JG, Bindels RJ: Cisplatin-induced injury of the renal distal convoluted tubule is associated with hypomagnesaemia in mice. *Nephrol Dial Transplant* 28: 879–889, 2013
111. Dimke H, van der Wijst J, Alexander TR, Meijer IM, Mulder GM, van Goor H, Tejpar S, Hoenderop JG, Bindels RJ: Effects of the EGFR inhibitor erlotinib on magnesium handling. *J Am Soc Nephrol* 21: 1309–1316, 2010

Published online ahead of print. Publication date available at www.cjasn.org.



Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion

I. David Weiner,^{*†} William E. Mitch,[‡] and Jeff M. Sands[§]

Abstract

Renal nitrogen metabolism primarily involves urea and ammonia metabolism, and is essential to normal health. Urea is the largest circulating pool of nitrogen, excluding nitrogen in circulating proteins, and its production changes in parallel to the degradation of dietary and endogenous proteins. In addition to serving as a way to excrete nitrogen, urea transport, mediated through specific urea transport proteins, mediates a central role in the urine concentrating mechanism. Renal ammonia excretion, although often considered only in the context of acid-base homeostasis, accounts for approximately 10% of total renal nitrogen excretion under basal conditions, but can increase substantially in a variety of clinical conditions. Because renal ammonia metabolism requires intrarenal ammoniogenesis from glutamine, changes in factors regulating renal ammonia metabolism can have important effects on glutamine in addition to nitrogen balance. This review covers aspects of protein metabolism and the control of the two major molecules involved in renal nitrogen excretion: urea and ammonia. Both urea and ammonia transport can be altered by glucocorticoids and hypokalemia, two conditions that also affect protein metabolism. Clinical conditions associated with altered urine concentrating ability or water homeostasis can result in changes in urea excretion and urea transporters. Clinical conditions associated with altered ammonia excretion can have important effects on nitrogen balance.

Clin J Am Soc Nephrol 10: 1444–1458, 2015. doi: 10.2215/CJN.10311013

Introduction

Nitrogen metabolism is necessary for normal health. Nitrogen is an essential element present in all amino acids; it is derived from dietary protein intake, is necessary for protein synthesis and maintenance of muscle mass, and is excreted by the kidneys. Under steady-state conditions, renal nitrogen excretion equals nitrogen intake. Renal nitrogen excretion consists almost completely of urea and ammonia. (To note, ammonia exists in two distinct molecular forms, NH_3 and NH_4^+ , which are in equilibrium with each other. In this review, we use the term *ammonia* to refer to the combination of both molecular forms. When referring to a specific molecular form, we state either NH_3 or NH_4^+ .) Other nitrogen compounds (e.g., nitric oxide metabolites, and nitrates) and many nitrogen-containing compounds (e.g., uric acid, urinary protein, etc.), comprise <1% of total renal nitrogen excretion. The two major components of renal nitrogen excretion, urea and ammonia, are regulated by a wide variety of conditions and play important roles in normal health and disease, including roles in the urine concentrating mechanism and in acid-base homeostasis. In this review, we discuss the mechanisms and regulation of both urea and ammonia handling in the kidneys, their roles in renal physiologic responses other than nitrogen excretion, and the clinical uses of urea production and metabolism.

Urea Introduction

Proteins throughout the body are continually turning over but at vastly different rates: consider the

short half-lives of transcription factors versus the longer half-lives of structural proteins of muscle. To achieve such differences, there must be biochemical mechanisms that precisely identify proteins to be degraded plus mechanisms that efficiently degrade doomed proteins. The consequence is that these processes do not interfere with the turnover of proteins that are required to maintain cellular functions. The “how” and “why” of the biochemical reactions that are required for maintenance of cellular functions are being uncovered (1,2). Here, we will examine the overall metabolism and functions of urea. Knowledge of urea functions and metabolism is important because urea is the major circulating source of nitrogen-containing compounds and it plays important roles in regulating kidney function.

Foods rich in protein are converted to the 9 essential and 11 nonessential amino acids, as shown in the summary of overall protein metabolism in Figure 1. The difference between the two groups is that the essential amino acids cannot be synthesized in the body and, hence, they must be provided in the diet or proteins cannot be synthesized. Amino acids have two fates: (1) they can be used to synthesize protein, or (2) they are degraded in a monotonous fashion in which the α -amino group is removed and converted to urea in the liver. Not surprisingly, the production of urea is closely related to the amount of protein eaten; therefore, urea can be used to estimate whether a patient with CKD is receiving the required amounts of protein (3,4). In addition, urea production serves as an estimate

^{*}Nephrology and Hypertension Section, North Florida/South Georgia Veterans Health System, Gainesville, Florida;

[†]Division of Nephrology, Hypertension, and Transplantation, University of Florida College of Medicine, Gainesville, Florida;

[‡]Nephrology Division, Baylor College of Medicine, Houston, Texas; and

[§]Nephrology Division, Emory University School of Medicine, Atlanta, Georgia

Correspondence:

Dr. I. David Weiner, Division of Nephrology, Hypertension, and Transplantation, University of Florida College of Medicine, P.O. Box 100224, Gainesville, FL 32610. Email: david.weiner@medicine.ufl.edu

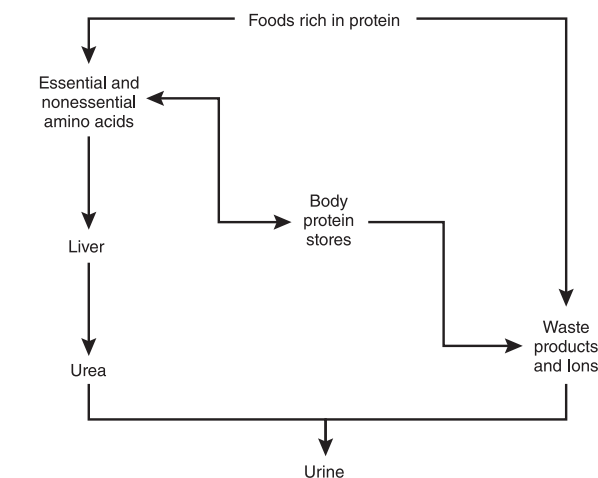


Figure 1. | Overview of protein metabolism. Dietary protein intake can either be metabolized quickly to essential and nonessential amino acids or to metabolic waste products and ions. Essential and nonessential amino acids are interconvertible with body protein stores. Amino acids may also be metabolized through the liver to form urea, which is then excreted in the urine. Body protein stores can be converted back to essential and nonessential amino acids or may be metabolized, forming waste products and ions, which, as previously detailed, are excreted in the urine.

of the accumulation of putative uremic toxins and, thus, as a guideline for management of the diets of patients with CKD. It has long been known that the amount of dietary protein affects renal function (5,6). For example, otherwise

normal individuals who eat small amounts of protein have low GFR values (7). On the contrary, eating a large meal of protein transiently raises GFR (8). The role of a dietary protein-induced change in GFR as a contributor to the consequences of CKD is unclear because most patients with advanced CKD eat substantially more protein than recommended by the World Health Organization (9).

Urea Transport

The urea transporter (UT)-A1 protein is expressed in the apical plasma membrane of the terminal inner medullary collecting duct (IMCD) (10–12). It consists of 12 transmembrane-spanning domains connected by a cytoplasmic loop (Figures 2 and 3) (13). UT-A3 is the N-terminal half of UT-A1 and is also expressed in the IMCD, primarily in the basolateral membrane, but can be detected in the apical membrane after vasopressin stimulation (14,15). UT-A2 is the C-terminal half of UT-A1 and is expressed in the thin descending limb (11,15–17). UT-A4 is the N-terminal 25% of UT-A1 spliced to the C-terminal 25% (11). UT-B1 protein is expressed in red blood cells (11,16,17) and in nonfenestrated endothelial cells that are characteristic of descending vasa recta, especially in those that are external to collecting duct clusters (18).

Urea Handling along the Nephron

Urea is filtered across the glomerulus and enters the proximal tubule. The concentration of urea in the ultrafiltrate is similar to plasma, so the amount of urea entering

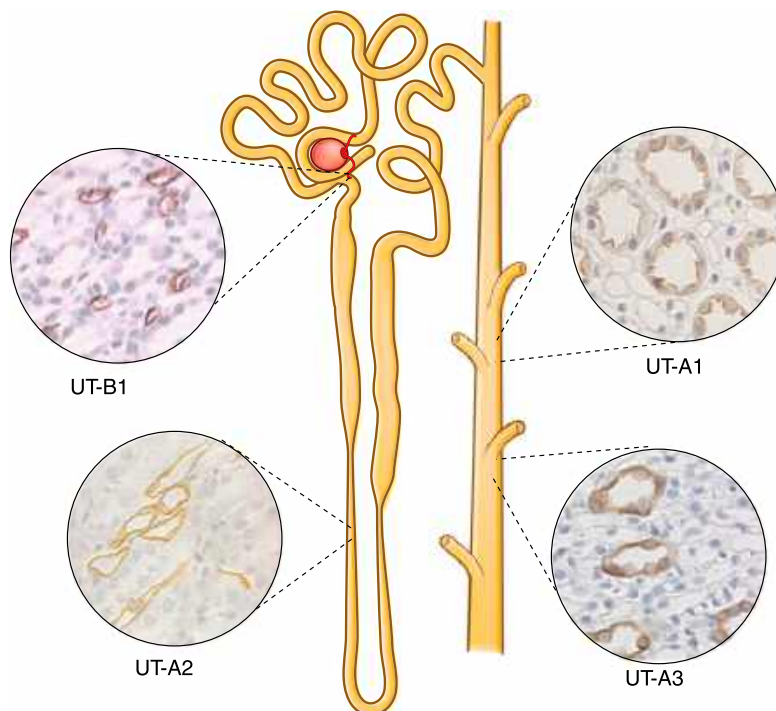


Figure 2. | Urea transporters along the nephron. The cartoon and histology show the urea transporters (UT-A1/UT-A3, UT-A2, and UT-B1) along the nephron. UT-B1 is found chiefly in the vasa recta, UT-A2 is found in the thin descending limb of the loop of Henle, and UT-A1 (apical) and UT-A3 (basolateral) are found in the inner medullary collecting duct. Modified from reference 12, with permission.

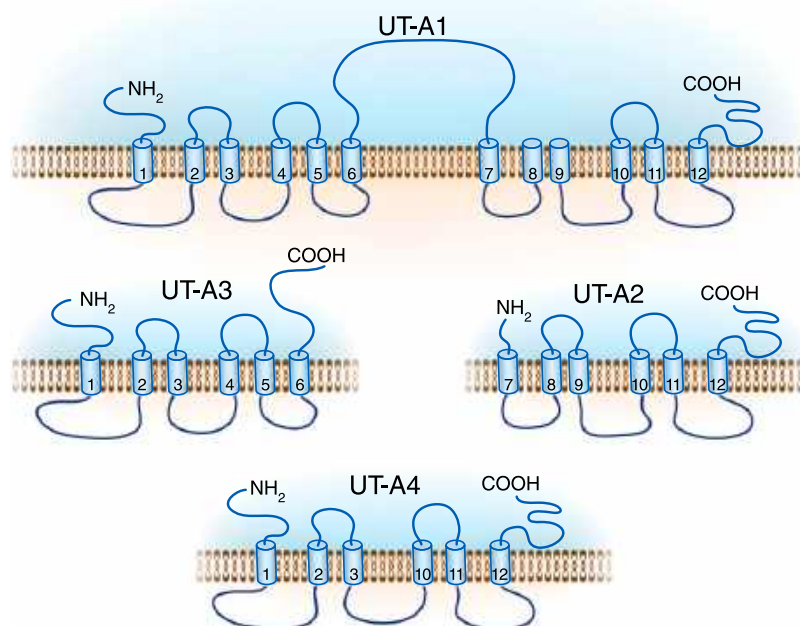


Figure 3. | The four renal UT-A protein isoforms. UT-A1 is the largest protein containing 12 transmembrane helices. Helices 6 and 7 are connected by a large intracellular loop that recent studies have shown is crucial to the functional properties of UT-A1 (1). UT-A3 is the N-terminal half of UT-A1, whereas UT-A2 is the C-terminal half of UT-A1. UT-A4 is the N-terminal quarter of UT-A1 spliced to the C-terminal quarter. Modified from reference 13, with permission.

the proximal tubule is controlled by the GFR. In general, 30%–50% of the filtered load of urea is excreted. The urea concentration increases in the first 75% of the proximal convoluted tubule, where it reaches a value approximately 50% higher than plasma (11). This increase results from the removal of water, secondary to salt transport, and is maintained throughout the remainder of the proximal tubule. Urea transport across the proximal tubule is not regulated by vasopressin (also named antidiuretic hormone) but is increased with an increase in sodium transport.

There are two types of loops of Henle: long looped in the juxtamedullary nephrons and short looped in the cortical nephrons. The difference is that short-looped nephrons lack a thin ascending limb (Figure 4). All portions of short loops are permeable to urea, but the direction and magnitude of urea movement varies with the diuretic state of the animal (11). The urea concentration in the early distal tubule (at the end of the loop) can reach 7 times the plasma concentration in antidiuretic rats, higher than the concentration at the start of the loop. Therefore, the intervening segments support urea secretion under antidiuretic conditions. By contrast, during water diuresis, there is no difference in the proximal tubular movement of urea, whereas there is net reabsorption of urea in the short loops (11).

Figure 5 summarizes urea permeabilities for the different nephron segments from rat kidney. The urea permeability of proximal convoluted tubules is higher than in proximal straight tubules. Thin descending limbs of short loops have a low urea permeability in the outer medulla, but there is a higher urea permeability in the long loops in the inner medulla. The increased intraluminal urea concentration in thin descending limbs results from a change

in the urea:water ratio because of water loss. Although there are considerable differences in the absolute urea permeability values measured in different animals, it is generally agreed that urea is secreted into the lumen of thin limbs under antidiuretic conditions (11). In addition, the concentration of urea is increased by water reabsorption driven by the hypertonic medullary interstitium, which results from the movement of urea out of the IMCD.

Urea concentration increases in thin ascending limbs (11) due to the gradient for urea secretion provided by urea reabsorption from the IMCD. The gradient decreases as thin ascending limbs ascend, and the driving force to move urea into the tubular lumen also decreases. The urea concentration reaches a level that is equi-osmolar with the surrounding interstitium by the beginning of the medullary thick ascending limb. In contrast with thin ascending limbs, thick ascending limbs have a lower urea permeability (11,16). However, there is an overall increase in urea concentration in the lumen from the beginning of the thick ascending limb to the distal convoluted tubule.

The distal convoluted tubule has a low urea permeability; however, some urea is reabsorbed in this segment so that the urea concentration decreases from approximately 110% of the filtered load to approximately 70% by the initial portion of the cortical collecting duct. Both the cortical and outer medullary collecting ducts have low urea permeabilities (11,16). By contrast, the IMCD has a high urea permeability, which is increased by vasopressin. There is extensive urea reabsorption from the IMCD lumen into the interstitium. The tubular fluid (urine) exiting the IMCD contains approximately 50% of the filtered load of urea.

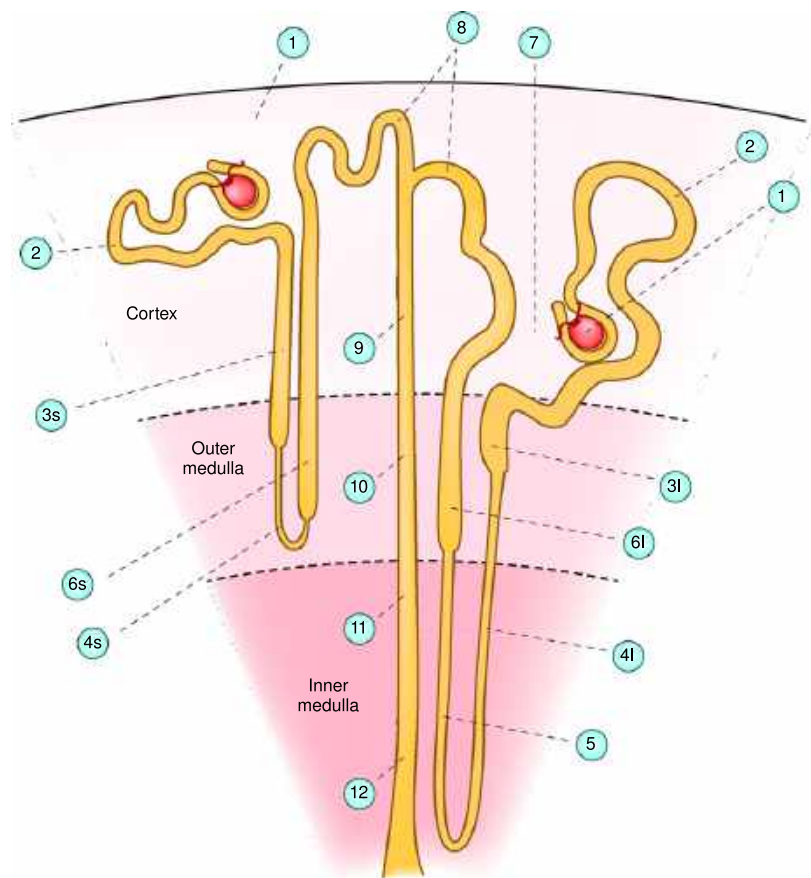


Figure 4. | Structure of the nephron. The cartoon depicts the cortex (top), outer medulla (middle), and inner medulla (bottom), showing the location of the various substructures of the nephron labeled as follows: 1, glomerulus; 2, proximal convoluted tubule; 3s and 3l, proximal straight tubule in the short-looped nephron (3s) and long looped nephron (3l); 4s and 4l, thin descending limb; 5, thin ascending limb; 6s and 6l, medullary thick ascending limb; 7, macula densa; 8, distal convoluted tubule; 9, cortical collecting duct; 10, outer medullary collecting duct; 11, initial inner medullary collecting duct; and 12, terminal inner medullary collecting duct. Modified from reference 11, with permission of the American Physiological Society.

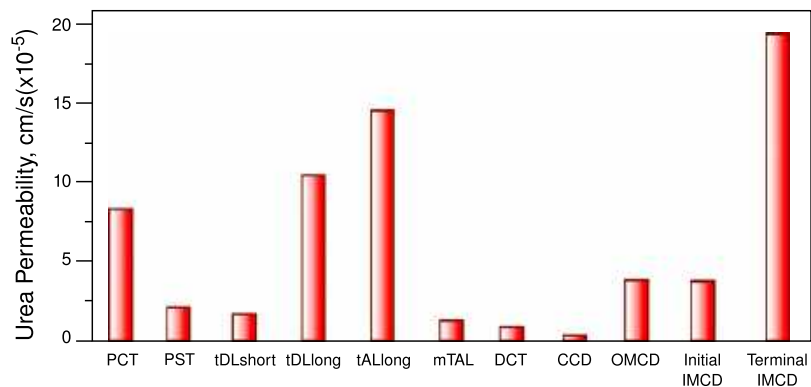


Figure 5. | Measured urea permeabilities in the different nephron sections of a rat kidney. CCD, cortical collecting duct; DCT, distal convoluted tubule; IMCD, inner medullary collecting duct; mTAL, medullary thick ascending limb; OMCD, outer medullary collecting duct; PCT, proximal convoluted tubule; PST, proximal straight tubule; TAL, thin ascending limb; tDL, thin descending limb. Modified from reference 11, with permission of the American Physiological Society.

Urine Concentrating Mechanism

Urea and urea transporters play key roles in the inner medullary processes for producing concentrated urine. Urea’s importance has been appreciated for nearly 8 decades, since Gamble *et al.* first described “an economy of

water in renal function referable to urea” (19). Protein deprivation reduces maximal urine concentrating ability and is restored by urea infusion or correction of the protein malnutrition (11,16). Decreased maximal urine concentrating ability is present in several genetically engineered mice

lacking different urea transporter(s), including UT-A1/A3, UT-A2, UT-B1, and UT-A2/B1 knockout mice (11,12,16). Thus, although the mechanism by which the inner medulla concentrates urine remains controversial, an effect derived from urea or urea transporters must play a role (11,16,17).

The most widely accepted mechanism for producing concentrated urine in the inner medulla is the passive mechanism hypothesis, proposed by Kokko and Rector (20) and Stephenson (21). The passive mechanism requires that the inner medullary interstitial urea concentration exceed the urea concentration in the lumen of the thin ascending limb. If an inadequate amount of urea is delivered to the deep inner medulla, urine concentrating ability is reduced because the chemical gradients necessary for passive NaCl reabsorption from the thin ascending limb cannot be established. The primary mechanism for urea delivery into the inner medullary interstitium is urea reabsorption from the terminal IMCD (22). Urea reabsorption is mediated by the UT-A1 and UT-A3 urea transporter proteins (11,16,17). Figure 2 shows the location of key urea transport proteins that are involved in urine concentration.

The UT-B1 urea transporter also plays an important role in urine concentration (11). UT-B1 protein is expressed in red blood cells and descending vasa recta (11). UT-B1 is the Kidd blood group antigen, a minor blood group antigen. People lacking UT-B1 are unable to concentrate their urine >800 mOsm/kg H_2O , even after water deprivation and vasopressin administration (23). UT-B1 knockout mice also have a reduced maximal urine concentrating ability compared with wild-type mice (24). These data suggest that urea transport in red blood cells is important for efficient countercurrent exchange, which is necessary for maximal urinary concentration (25). As red blood cells descend into the medulla, they accumulate urea to stay in osmotic

equilibrium with the medullary interstitium. As the red blood cells ascend in the ascending vasa recta, they need to lose urea. In the absence of UT-B1, the red blood cells are unable to lose urea quickly enough and take some of the urea out of the medulla and into the bloodstream, thereby reducing the efficiency of countercurrent exchange and urine concentrating ability (25).

Rapid Regulation of Urea Transporter Proteins

Vasopressin. Vasopressin regulates both IMCD urea transporters UT-A1 and UT-A3. Vasopressin increases the phosphorylation and apical plasma membrane accumulation of UT-A1 and UT-A3 (14,26). Vasopressin phosphorylates serines 486 and 499 in UT-A1, and both must be mutated to eliminate vasopressin stimulation (27). Vasopressin increases urea transport, urea transporter phosphorylation, and apical plasma membrane accumulation through two cAMP-dependent pathways: protein kinase A and exchange protein activated by cAMP (28) (Figure 6). Genetically engineered mice lacking UT-A1 and UT-A3 have reduced urine concentrating ability, have reduced inner medullary interstitial urea content, and lack vasopressin-stimulated urea transport in their IMCDs (29).

Hypertonicity. Urea transport across the IMCD is regulated independently by hypertonicity (11,16,17). Urea permeability increases rapidly in perfused IMCDs when osmolality is increased, even in the absence of vasopressin (30,31). Vasopressin and hyperosmolality have an additive stimulatory effect on urea permeability (11,16,17). Hyperosmolality, similar to vasopressin, increases the phosphorylation and the plasma membrane accumulation of both UT-A1 and UT-A3 (14,26,32,33). However, hyperosmolality and vasopressin signal through different pathways: hyperosmolality *via* increases in protein kinase $C\alpha$ and

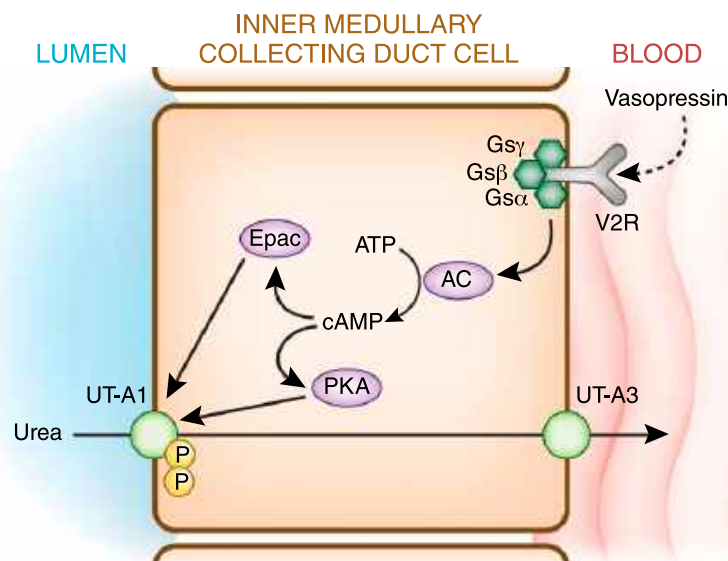


Figure 6. | Urea transport across an IMCD cell. Vasopressin binds to the V_2R , located on the basolateral plasma membrane, and activates the α subunit of the heterotrimeric G protein $G_{s\alpha}$. Activation of the G protein stimulates AC to synthesize cAMP. The increase of intracellular cAMP stimulates several downstream proteins including PKA and Epac, which phosphorylate UT-A1 and increase its accumulation in the apical plasma membrane. Urea enters the IMCD cell through UT-A1 and exits on the basolateral plasma membrane *via* UT-A3. AC, adenylate cyclase; Epac, exchange protein directly activated by cAMP; Gs, G protein stimulatory subunit; P, phosphate; PKA, protein kinase A; V_2R , V_2 vasopressin receptor. Modified from reference 13 with permission.

intracellular calcium, and vasopressin *via* increases in adenylyl cyclase (11,16,17). Hyperosmolality does not stimulate urea transport in protein kinase C α knockout mice and they have a urine concentrating defect (31,34,35).

Long-Term Regulation of Urea Transporters

Vasopressin. Vasopressin regulates the IMCD urea transporters in the long term through changes in protein abundance (11,16,17). Administering vasopressin for 2 weeks to rats that have a congenital lack of vasopressin, and, hence, central diabetes insipidus, increases UT-A1 protein abundance in the inner medulla (36). Suppressing endogenous vasopressin by water loading rats for 2 weeks decreases UT-A1 protein abundance (36). The increase in UT-A1 protein abundance after vasopressin administration matches the time course for the increase in inner medullary urea content after vasopressin administration to rats that have a congenital lack of vasopressin. UT-A3 protein expression is decreased in rats that are water loaded for 3 days and is increased in rats that are water restricted for 3 days. The effect of vasopressin on UT-B1 abundance is unclear because some studies show an increase and others show a decrease (11,16,17).

Low-Protein Diets. Rats fed a low-protein diet for at least 2 weeks have a decrease in the fractional excretion of urea (37). This results, at least in part, from the functional expression of vasopressin-stimulated urea permeability in the initial IMCD, a segment in which it is not normally present, and an increase in UT-A1 protein abundance (11,16,17). The effect of a low-protein diet on the other urea transporters has not been studied.

Adrenal Steroids. Glucocorticoids increase the fractional excretion of urea (38). Despite this increase, serum urea nitrogen (SUN) increases in patients given glucocorticoids. This indicates that the increase in urea excretion is insufficient to offset the increase in production in patients given glucocorticoids.

Adrenalectomy, which eliminates both glucocorticoids and mineralocorticoids, produces a urine concentrating defect, although the mechanism is unknown (11). Adrenalectomy increases UT-A1 protein abundance and urea permeability in rat terminal IMCDs (39). Administering dexamethasone to adrenalectomized rats decreases UT-A1 protein abundance and urea permeability (39). Both UT-A1 and UT-A3 protein abundances decrease in rats given dexamethasone (40). The decrease in urea transporters in dexamethasone-treated rats could explain the increase in the fractional excretion of urea because a reduction in urea transporter abundance could result in less urea being reabsorbed and, thus, more being excreted.

Administering mineralocorticoids to adrenalectomized rats also decreases UT-A1 protein abundance in the inner medulla (41). This decrease can be blocked by spironolactone, a mineralocorticoid receptor antagonist (41). Both mineralocorticoid and glucocorticoid hormones appear to work through their respective receptors because spironolactone does not block the decrease due to dexamethasone (41).

Acidosis. Metabolic acidosis is a common complication of renal failure. Acidosis increases protein degradation and shifts the nitrogen and urea loads within the kidney (42). UT-A1 protein abundance increases in the inner medulla of acidotic rats, which may represent compensation for the

loss of kidney concentrating ability (increase in urine volume and a decrease in urine osmolality) that occurs during acidosis (43).

Hypokalemia. Prolonged hypokalemia can cause a decrease in urine concentrating ability (44). The abundance of UT-A1, UT-A3, and UT-B1 proteins in the inner medulla is reduced in rats fed a potassium-restricted diet (44,45). UT-A2 protein abundance was reduced in one study but increased in another (44,45). The reason for the different findings is unclear.

In summary, renal urea transport and urea transport proteins mediate a central role in the urine concentrating mechanism. Urine concentrating defects have been demonstrated in several urea transporter knockout mice (11,12,16). In many clinical conditions associated with altered urine concentrating ability or water homeostasis, changes in urea excretion and urea transporters may be contributory factors.

Ammonia

Physiologic Role for Ammonia

Kidneys mediate a central role in acid-base homeostasis through the combined functions of filtered bicarbonate reabsorption and new bicarbonate generation. Bicarbonate reabsorption is necessary for acid-base homeostasis, but it is not sufficient. New bicarbonate must be generated to replace the bicarbonate that buffered endogenous and exogenous acids. New bicarbonate generation involves urinary ammonia and titratable acid excretion. Ammonia excretion accounts for the majority of basal bicarbonate generation and changes in ammonia excretion are the primary response to acid-base disorders (Figure 7). Nitrogen excretion in the form of ammonia is approximately 10% of urea nitrogen excretion in basal conditions, but can increase 5- to 10-fold, enabling ammonia to have an important role in nitrogen balance.

Renal Ammonia Handling

Overview. Renal ammonia metabolism differs in important ways from that of other renal solutes. Other renal solutes undergo net excretion, such that renal venous content is less than arterial content. Ammonia is fundamentally different. Almost all urinary ammonia is produced in the kidney (47), and renal venous ammonia exceeds arterial ammonia, meaning that the kidneys actually increase systemic ammonia. Ammonia undergoes a complex set of transport events in the kidney, which determines the proportion of ammonia generated that is excreted in the urine as ammonia nitrogen versus that which enters the renal capillaries and is transported to the systemic circulation through the renal veins.

Renal Ammoniogenesis. Renal ammoniogenesis occurs primarily in the proximal tubule and glutamine is the primary substrate (48). In the proximal tubule, glutamine uptake occurs through the combination of the apical Na⁺-dependent neutral amino acid transporter-1, and the basolateral sodium-coupled neutral amino acid transporter-3 (SNAT3) (49). Changes in ammoniogenesis, such as during metabolic acidosis, are associated with changes in the expression of SNAT3, but not expression of apical Na⁺-dependent neutral amino acid transporter-1 (50). Ammoniogenesis primarily

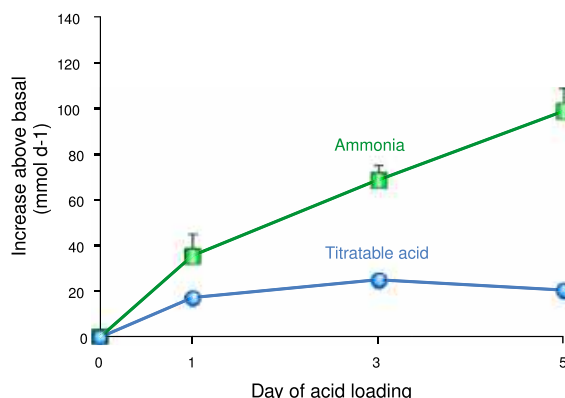


Figure 7. | Responses of urinary ammonia and titratable acid excretion to exogenous acid loads. Normal humans were acid loaded, and changes in urinary ammonia and titratable acid excretion were determined on days 1, 3, and 5 of acid loading. Changes in urinary ammonia excretion are the quantitatively predominant response mechanism on each day, and continued to increase over the 5 days of the experiment. Titratable acid excretion is a minor component of the increase in net acid excretion, and peaks on day 1 of acid loading. Data calculated from reference 46.

involves phosphate-dependent glutaminase, glutamate dehydrogenase, α -ketoglutarate dehydrogenase, and phosphoenolpyruvate carboxykinase (PEPCK) (47,51), and complete glutamine metabolism generates two NH_4^+ and two HCO_3^- ions per glutamine. The bicarbonate produced is then transported across the basolateral membrane *via* electrogenic sodium-coupled bicarbonate co-transporter, isoform 1A (NBCe-1A), and serves as “new bicarbonate” generated by the kidney.

Ammonia Transport Overview

Only approximately 50% of the ammonia produced is excreted in urine under basal conditions. The remaining ammonia enters the systemic circulation through the renal veins. Ammonia that enters the systemic circulation undergoes almost complete metabolism in the liver; the major metabolic pathway uses HCO_3^- as a substrate, and generates urea. Consequently, ammonia produced in the kidney, transported to the systemic circulation, and metabolized in the liver to urea has no net acid-base benefit.

The proportion of the ammonia produced that is excreted in the urine, as opposed to being transported into the systemic circulation, can be rapidly altered. This enables changes in urinary ammonia to exceed, at least acutely, changes in ammoniogenesis (52). In most chronic acid-base disturbances, changes in ammoniogenesis account for the majority of the changes in urinary ammonia content (47). Importantly, renal epithelial cell ammonia transport determines the proportion of ammonia excreted in the urine.

Ammonia Transport. Ammonia produced in the proximal tubule is secreted preferentially into the luminal fluid (Figure 8). This appears to involve NH_4^+ secretion by the apical NHE3; there may also be a component of parallel H^+ and NH_3 secretion (53–55). Conditions associated with NHE3 activation, such as metabolic acidosis and hypokalemia, also increase ammonia secretion (54).

In the loop of Henle, ammonia reabsorption occurs, with the major transport site being the medullary thick ascending limb. Ammonia is reabsorbed in the form of NH_4^+ primarily *via* the apical, loop diuretic-sensitive transporter, NKCC2 (Figure 9). Although other NH_4^+ transporters are present, their quantitative contribution is much less. Ammonia is then transported across the basolateral membrane, largely *via* the basolateral sodium-hydrogen exchanger NHE4 (56). NH_4^+ transport across the apical membrane, because NH_4^+ is a weak acid, causes intracellular acidification (57) which can inhibit ammonia reabsorption; bicarbonate entry *via* the basolateral electroneutral sodium-bicarbonate cotransporter, isoform 1 (NBCn1) appears to buffer this intracellular acidification and enable continued ammonia reabsorption (58). The net result is an axial interstitial ammonia gradient, with the highest levels in the inner medulla, the intermediate levels in the outer medulla and the lowest levels in the renal cortex.

Ammonia is then secreted by the collecting duct (Figure 10). Collecting duct ammonia secretion involves parallel H^+ and NH_3 secretion (59). NH_3 secretion appears to involve transport by the Rhesus glycoproteins Rhbg and Rhcg, ammonia-specific transporters expressed in the collecting duct (60–62). Basolateral $\text{Na}^+\text{-K}^+\text{-ATPase}$ contributes to IMCD ammonia secretion through its ability to transport NH_4^+ (63). Apical H^+ secretion involves both $\text{H}^+\text{-ATPase}$ and $\text{H}^+\text{-K}^+\text{-ATPase}$.

An important recent addition to our understanding of ammonia transport is the identification that sulfatides (highly charged, anionic glycosphingolipids) are important for maintaining papillary ammonium concentration and for urinary ammonia excretion during metabolic acidosis (64). Their expression is highest in the inner medulla, intermediate in the outer medulla, and lowest in the cortex. They appear to bind NH_4^+ reversibly, facilitating development of the axial ammonia gradient and ammonia excretion. Figure 11 shows an integrated view of renal ammonia metabolism.

Regulation of Ammonia Metabolism

Metabolic Acidosis. The primary mechanism by which the kidneys increase net acid excretion in response to metabolic acidosis is through increased ammonia metabolism. Almost every component of ammonia metabolism is increased, including SNAT3, phosphate-dependent glutaminase, glutamate dehydrogenase, PEPCK, NKCC2, NHE4, Rhbg, and Rhcg (51). This integrated response increases renal glutamine uptake, ammoniogenesis, generation of new bicarbonate, and ammonia excretion in urine.

To understand the effect of ammonia metabolism on nitrogen balance, it is important to consider the metabolic source of the glutamine used for ammoniogenesis. Renal ammoniogenesis averages approximately 60–80 mmol/d in humans under basal conditions, and can increase, assuming renal ammonia excretion is approximately 50% of total ammonia production, to approximately 3–400 mmol/d. Because each glutamine metabolized generates two NH_4^+ molecules, approximately 150–200 mmol/d of glutamine is necessary to support maximal rates of ammoniogenesis. During metabolic acidosis, acidosis stimulates skeletal muscle protein degradation that, coupled with hepatic glutamine synthesis, increases extrarenal glutamine production. This enables unchanged plasma

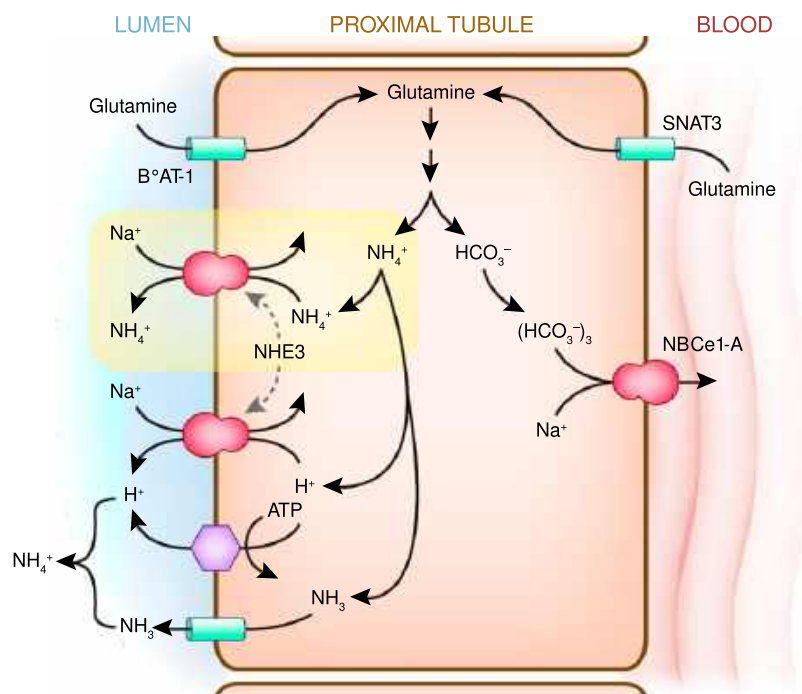


Figure 8. | Model of proximal ammonia transport. Glutamine serves as the primary metabolic substrate for ammoniogenesis. Proximal tubule glutamine uptake involves transport across the apical membrane, primarily *via* B⁰AT-1, and across the basolateral membrane by SNAT3. Complete metabolism of each glutamine results in generation of two NH₄⁺ and two bicarbonate ions. Bicarbonate is transported across the basolateral membrane *via* NBCe-1A. Ammonium secretion across the apical membrane occurs primarily *via* NHE3-mediated Na⁺/NH₄⁺ exchange, with a lesser contribution by parallel H⁺ and NH₃ transport. B⁰AT-1, apical Na⁺-dependent neutral amino acid transporter-1; NBCe-1A, electroneutral sodium-bicarbonate cotransporter, isoform 1A; NHE3, sodium/hydrogen exchanger 3; SNAT3, sodium-coupled neutral amino acid transporter-3.

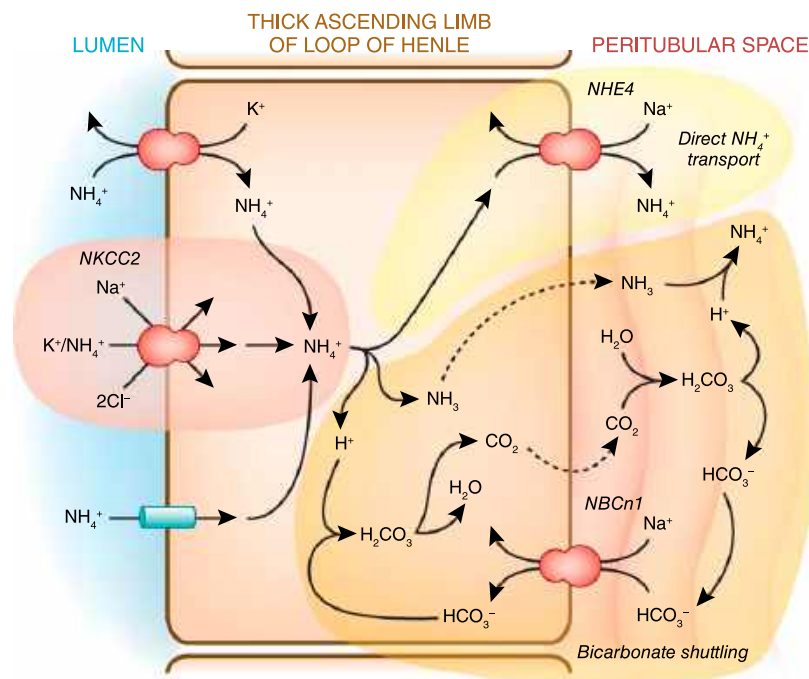


Figure 9. | Ammonia reabsorption by the thick ascending limb. Primary mechanism of apical ammonium absorption is *via* substitution of NH_4^+ for K^+ and transport by the loop diuretic-sensitive, apical NKCC2 transporter. Cytoplasmic NH_4^+ is transported across the basolateral membrane either *via* $\text{Na}^+/\text{NH}_4^+$ exchange mediated by NHE4 or *via* a bicarbonate shuttling mechanism involving NH_3 transport. NBCn1, electroneutral sodium bicarbonate cotransporter, isoform 1; NHE4, sodium/hydrogen exchanger 4.

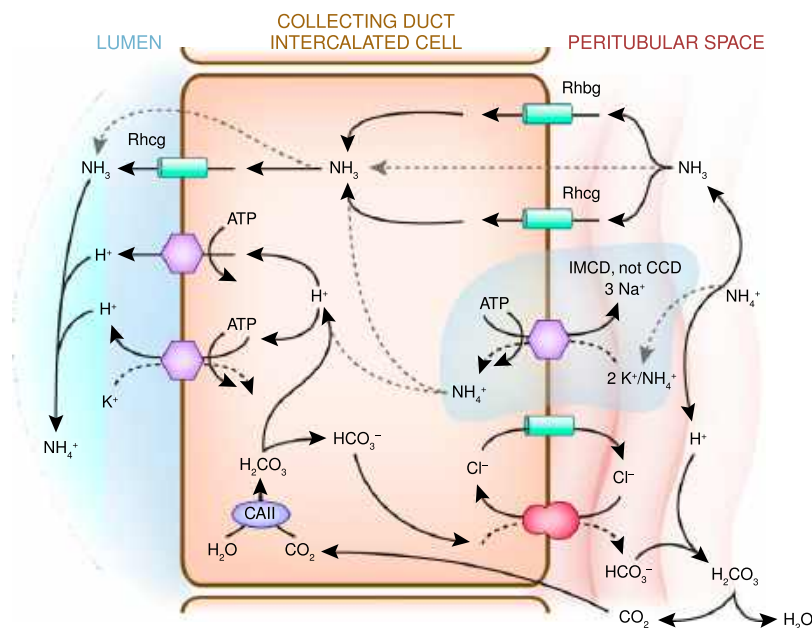


Figure 10. | Ammonia secretion by the collecting duct. Ammonia uptake across the basolateral membrane primarily involves either transporter-mediated uptake across the basolateral membrane by Rhb or Rhc, with a component of diffusive NH_3 absorption. Cytosolic NH_3 is transported across the apical membrane by a combination of Rhc and diffusive transport. In the IMCD, but not the CCD, basolateral Na^+/K^+ -ATPase also contributes to NH_4^+ uptake across the basolateral membrane. Cytosolic H^+ is generated by a carbonic anhydrase II-mediated mechanism, and is secreted across the apical membrane via H^+ -ATPase and H^+/K^+ -ATPase. Luminal H^+ titrates luminal NH_3 , forming NH_4^+ and maintaining a low luminal NH_3 concentration necessary for NH_3 secretion. CAII, carbonic anhydrase isoform II; Rhb, Rhesus B glycoprotein; Rhc, Rhesus C glycoprotein.

glutamine levels despite substantial increases in renal glutamine uptake (65).

This role of skeletal muscles in metabolic acidosis has important clinical significance. Metabolic acidosis decreases skeletal muscle mass and it increases ammonia nitrogen excretion, which can cause negative nitrogen balance (65). Correction of the metabolic acidosis associated with CKD improves nitrogen balance, plasma albumin, skeletal muscle size, and skeletal muscle strength (66,67).

Hypokalemia. Hypokalemia is a second condition associated with altered renal ammonia metabolism. Indeed, the increased bicarbonate generation contributes to the metabolic alkalosis often seen with hypokalemia. In addition, in adults on an otherwise adequate, but low-protein, diet, hypokalemia-induced increases in ammonia excretion can cause negative nitrogen balance (68). In children with a low but otherwise adequate protein intake, hypokalemia reduces the total body nitrogen retention necessary for normal protein synthesis and impairs growth due to increased nitrogen excretion in the form of ammonia (68).

Glucocorticoid Hormones. Glucocorticoid hormones regulate approximately 70% of basal and 50%–70% of acidosis-stimulated ammonia excretion (69,70). Their role appears to involve regulation of SNAT3, PEPCK, and NHE3 (71–74). In addition, glucocorticoids contribute to acidosis-induced skeletal muscle protein degradation (65), which by contributing to extrarenal glutamine production, enables maintenance of normal plasma glutamine levels (70). Thus, glucocorticoid hormones have an important role in nitrogen balance mediated, in part, through their effects on ammonia metabolism.

Protein Intake. Dietary protein intake has important effects on renal ammonia metabolism. In general, high-protein

diets, particularly if high in sulfur-containing amino acids, increase endogenous acid production, causing a parallel increase in ammonia excretion, whereas low-protein diets decrease ammonia excretion (75,76). Because ammonia nitrogen excretion changes parallel dietary nitrogen changes, net nitrogen balance does not change.

However, the clinician should remember that urinary ammonia averages only approximately 50% of total renal ammonia production, and that a similar amount enters the systemic circulation *via* the renal veins. Thus, after protein intake, increased renal vein ammonia content can increase plasma ammonia levels (77). In patients with impaired hepatic function, this can either precipitate or worsen hepatic encephalopathy. Similarly, the protein load from red cell breakdown resulting from gastrointestinal bleeding can increase renal ammoniogenesis, leading to increased renal vein ammonia, which may contribute to development or worsening of hepatic encephalopathy (78).

Urea Production and Metabolism

Clinical Uses

Uremic symptoms are principally due to the accumulation of ions and toxic compounds in body fluids (79). Because protein-rich foods are the major source of these waste products, CKD can be considered a condition of protein intolerance. Indeed, it has been known since at least 1869 that restricting the amount of protein in the diet of patients with kidney diseases improves their uremic symptoms (80). More recently, we learned that dialysis efficacy is reflected in the removal of urea because changes in urea accumulation reflect changes in accumulated metabolic

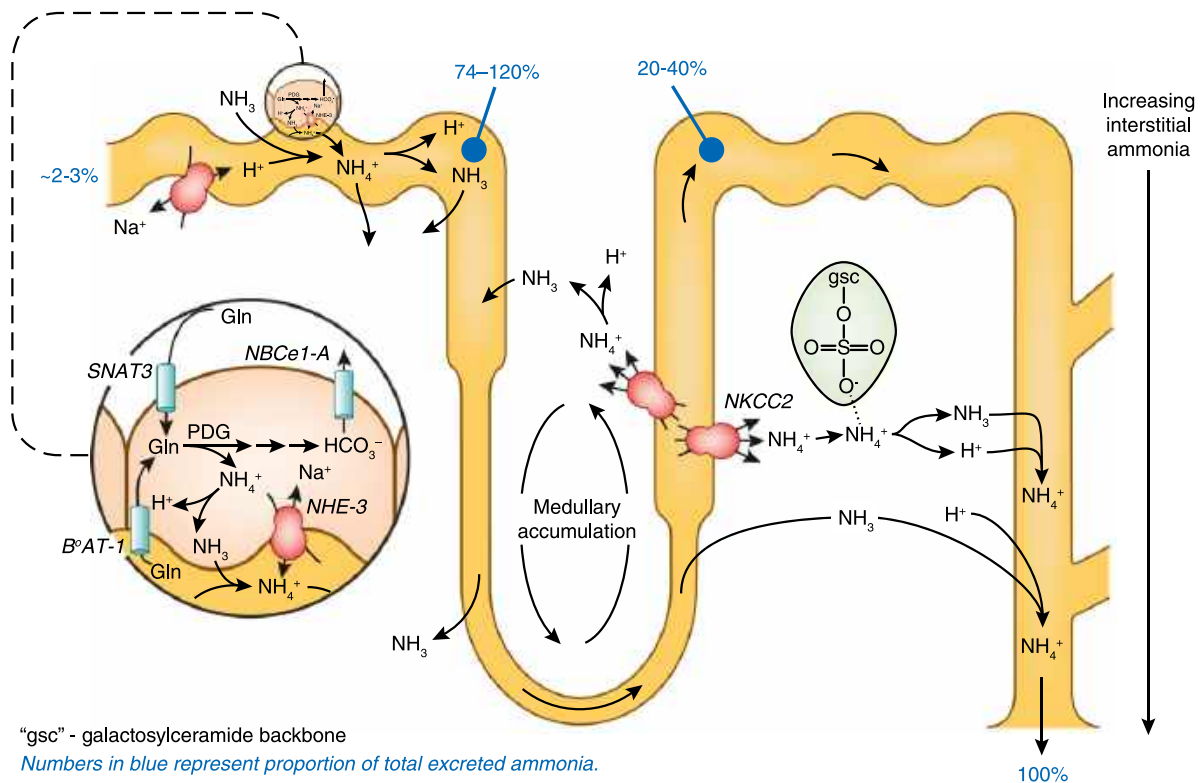


Figure 11. | Integrated overview of renal ammonia metabolism. Renal ammoniogenesis occurs primarily in the proximal tubule, involving glutamine uptake by SNAT3 and B⁰AT-1, glutamine metabolism forming ammonium and bicarbonate, and apical NH₄⁺ secretion involving NHE3 and parallel H⁺ and NH₃ transport. Ammonia reabsorption in the thick ascending limb, involving apical NKCC2-mediated uptake results in medullary ammonia accumulation. Medullary sulfates (highlighted in green) reversibly bind NH₄⁺, contributing to medullary accumulation. Ammonia is secreted in the collecting duct via parallel H⁺ and NH₃ secretion. The numbers in blue represent the proportion of total excreted ammonia. B⁰AT-1, apical Na⁺-dependent neutral amino acid transporter-1; gsc, galactosylceramide backbone; PDG, phosphate-dependent glutaminase.

waste products. Again, this is not a new concept: The link between dietary protein and urea has been recognized since at least 1905, when Folin reported that urea excretion varies directly with different levels of dietary protein (81). These relationships were elegantly documented by Cottini *et al.* (Figure 12), who fed patients with CKD different amounts of protein (expressed on the abscissa as nitrogen intake because 16% of protein is nitrogen) (82). With low levels of dietary protein (*e.g.*, approximately 12 g protein/d equivalent to approximately 2.5 g nitrogen), nitrogen balance was negative, indicating that this level of dietary protein causes progressive loss of protein stores. When the diet was raised >4 g nitrogen/d, nitrogen balance became positive, signifying that protein stores were being maintained. With progressively more dietary protein, nitrogen balance remained positive but changed minimally. Instead, when dietary protein was above the level required to maintain nitrogen balance and protein stores, it was used to make urea. Clearly, urea production reflects the level of protein in the diet and the risk of developing complications of uremia. In addition, a high-protein diet invariably contains excesses of salt, potassium, phosphates, and so forth (83). The clinical problems that arise from high-protein diets in patients with CKD were recently highlighted in reports concluding that increases in salt intake or serum phosphorus will block the beneficial influence of angiotensin-converting enzyme inhibitors to delay the progression of CKD (84,85).

Urea has special properties that can be used to evaluate the severity of uremia or the degree of compliance with prescribed changes in the diet. These properties include the following: (1) a very large capacity for hepatic urea production from amino acids, (2) urea is the major circulating pool of nitrogen and it crosses cell membranes readily so there is no gradient from intracellular to extracellular fluid under steady-state conditions, and (3) the volume of distribution of urea is the same as water (the urea space is estimated as 60% of body weight) (86–88).

One clinically useful calculation is the steady-state SUN (S_{SUN}), which reflects the severity of uremia because it estimates the degree of accumulation of protein-derived waste products. The S_{SUN} calculation is useful because uremic symptoms are unusual when S_{SUN} is <70 mg/dl.

The requirements for the calculation are that the patient with CKD is in the steady state (*i.e.*, his or her SUN and weight are stable) and urea clearance in liters per day is known. Using the equation below, the amount of dietary protein that will yield a S_{SUN} of 70 mg/dl can be calculated as:

$$S_{\text{SUN}} = (\text{dietary protein} \times 0.16 - 0.031 \text{ g nitrogen/kg per day} \times \text{weight}) / \text{urea clearance in L/d.}$$

The following steps are used to calculate the S_{SUN}. First, the prescribed dietary protein in grams per day is converted

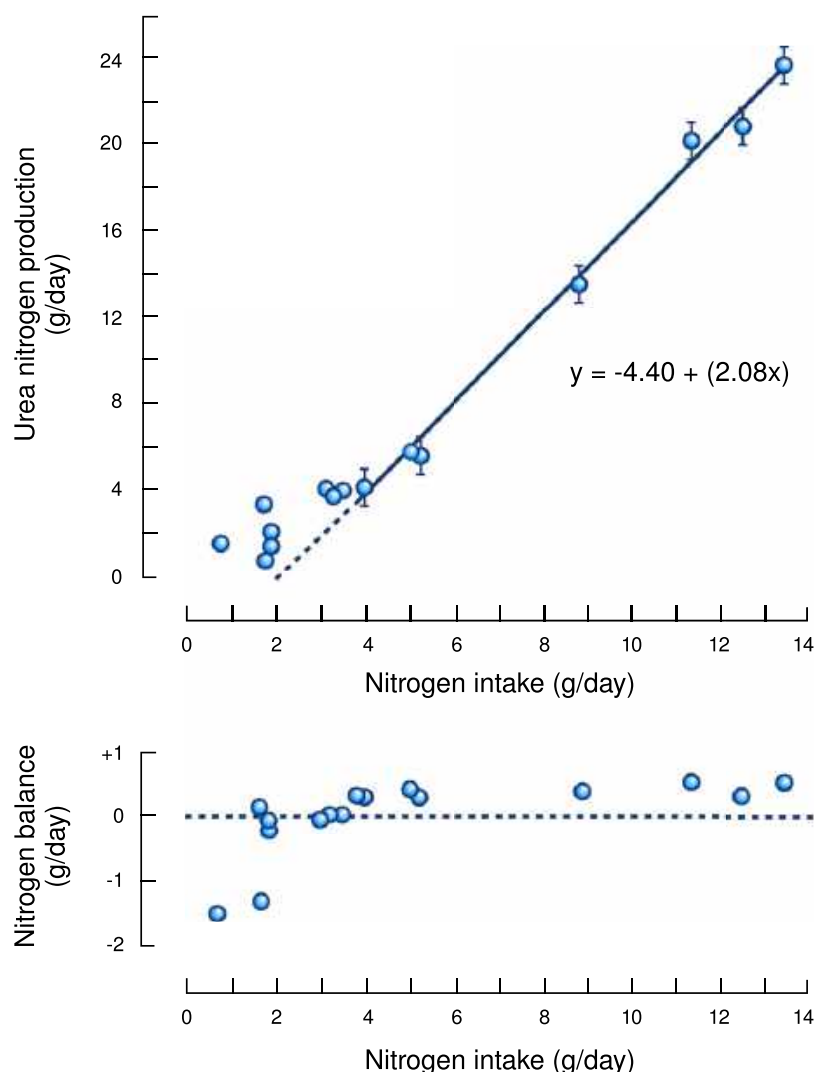


Figure 12. | Urea excretion in adult humans with varying degrees of kidney malfunction fed milk, egg, or an amino acid mixture: assessment of nitrogen balance. Modified from reference 82, with permission.

into dietary nitrogen by multiplying the grams per day of dietary protein by 16%. Second, the nonurea nitrogen in grams of nitrogen excreted per day is calculated as the excretion of all forms of nitrogen except urea. This amount is approximated as 0.031 g nitrogen/kg per day multiplied by the nonedematous, ideal body weight (4,89). Third, the nonurea nitrogen is subtracted from the nitrogen intake to obtain the amount of urea nitrogen that must be excreted each day in the steady state. Finally, dividing the urea nitrogen excretion in grams per day by the urea clearance in liters per day yields the S_{SUN} in grams per liter.

For example, consider a 70-kg adult with a urea clearance of 14.4 L/d (or 10 ml/min) who is eating 76 g protein/d. His S_{SUN} (in grams per liter) is calculated from the following: 12.2 g/d dietary nitrogen – (0.031 g nitrogen/kg per day times 70 kg). The result is divided by the urea clearance in liters per day and multiplied by 100 to convert S_{SUN} 0.69 g/L to 69 mg/dl.

This calculation arises from the demonstration that in the steady state, the production of urea is directly proportional

to the daily protein intake (Figure 12). The only other assumption is that urea clearance is independent of the plasma urea concentration, which is reasonable for patients with CKD. The key concept is that steady-state concentrations of nitrogen-containing waste product produced during protein catabolism will increase in parallel to an increase in the S_{SUN} (4,82,89). By varying the amount of dietary protein, changes in the diet can be integrated with different values of the S_{SUN} . As shown in Table 1, similar concepts can be used to determine whether a patient is complying with the prescribed protein content of the diet (81,86,88).

These examples emphasize that the net production of urea in patients with CKD (also known as the urea appearance rate) can be used to estimate protein intake (4,82,89). For dialysis patients, the same relationships have been labeled as “urea generation” or the “normalized protein catabolic rate” (nPCR). Obviously, the nPCR equals the net urea production rate or the urea appearance rate except that it is not expressed per kilogram of body

weight. However, the designation nPCR is misleading because the rates of protein synthesis and “catabolism” are far greater than the protein catabolic rate: The nitrogen flux in protein synthesis and degradation amounts to 45–55 g nitrogen/d, equivalent to 280–350 g protein/d (1). The principle of conservation of mass, however, indicates the difference between whole-body protein synthesis and degradation does estimate waste nitrogen production.

Urea Nitrogen Reutilization

Discussion of urea metabolism would be incomplete without addressing urea degradation. It is calculated from the plasma disappearance of injected [¹⁴C]urea or [¹⁵N] urea (88,90) and averages about 3.6 g nitrogen/d in both normal individuals and patients with uremia. The 3.6 g nitrogen/d arises from degradation of urea by ureases of gastrointestinal bacteria thereby supplying ammonia directly to the liver (88). Because this source of nitrogen could be used to synthesize amino acids and ultimately protein, the degradation of urea has been intensively studied (91). The evidence negates the hypothesis that urea degradation is nutritionally important. First, the amount of urea degraded has been expressed as an extrarenal urea clearance by dividing the rate of urea degradation by the S_{SUN}. In normal adults, the extrarenal urea clearance averages approximately 24 L/d; if this value were present in patients with CKD and a high SUN, the amount of ammonia derived from urea would be very high (79,88). However, the quantity of ammonia arising from urea in patients with CKD is not significantly different from that of normal individuals, indicating that the extrarenal clearance of urea in patients with CKD must be greatly reduced; the mechanism for this observation is unknown (88).

Results from other testing strategies lead to the conclusion that it is unlikely that urea degradation contributes a nutritionally important source of amino acids to synthesize protein. We fed patients with CKD a protein-restricted diet and measured the turnover of urea using [¹⁴C]urea. The results were compared with those obtained in a second experiment in which patients received neomycin/kanamycin as nonabsorbable antibiotics in order to inhibit bacteria that were degrading urea. In roughly half of the patients, antibiotic administration blocked urea degradation but there was no associated increase in urea appearance. This result means that ammonia arising from degradation

is simply recycled into urea production and hence does not change urea appearance (90). We also addressed the hypothesis that removal of nitrogen released by urea degradation would suppress synthesis of amino acids and thereby worsen Bn. In this case, the hypothesis was rejected because inhibiting urea degradation with nonabsorbable antibiotics actually improved Bn (92). Finally, Varcoc *et al.* measured the turnover of urea and albumin simultaneously and concluded that the contribution of urea degradation to albumin synthesis was minimal (93).

The possibility that ammonia from urea degradation is used to synthesize amino acids was recently examined in hibernating bears (94). The authors noted that hibernating bears have very low values of S_{SUN} (approximately 5–10 mg/dl) despite a decrease in GFR and they suggested that S_{SUN} was low because urea was being used to synthesize amino acids. This finding would contribute to another oddity of hibernating bears, namely that their muscle mass and other stores of protein are relatively “spared” from degradation. Why the metabolism of hibernating bears might differ from that of patients with CKD is unknown and we applaud the investigators who gathered the information as experimenting on bears is quite tricky, even if they are hibernating.

Is Urea Toxic?

Because excess dietary protein produces uremic symptoms and because urea is the major source of circulating nitrogen, the potential for toxic responses to urea have been investigated using different experimental designs. Johnson *et al.* added urea to the dialysate of hemodialysis patients who were otherwise well dialyzed (95). Complications induced by the added urea were minimal until the S_{SUN} was chronically >150–200 mg/dl. This led to gastrointestinal irritation. There was no investigation of ammonia or inhibitors of urea degradation so the effect of urea can only be considered an association. An indirect evaluation of both mice with CKD and cultured cells revealed that urea may stimulate the production of reactive oxygen species. Reddy *et al.* concluded that a high SUN not only increased reactive oxygen species but also caused insulin resistance (96). However, it is difficult to assign insulin resistance to a single factor considering that there are so many uremia-induced complex metabolic pathways (97,98). It will be interesting to evaluate whether the production of

Table 1. Estimation of protein intake from urea metabolism
A 60-year-old man with stage 5 CKD is admitted to the hospital for plastic surgery. He weighs 70 kg and has been taught to follow a diet containing 40 g protein/d (6.4 g nitrogen/d because protein is 16% nitrogen). He excretes 4 g urea nitrogen/d, but on day 2 his BUN rises from 50 to 60 mg/dl. • The increase in BUN signifies accumulation of urea nitrogen in body water (70 kg x 0.6 L/kg x 0.1 g urea nitrogen/L = 4.2 g urea nitrogen/d). • His NUN is 70 kg x 0.031 g nitrogen/kg per day = 2.17 g nitrogen/d. • The total nitrogen excreted and accumulated is approximately 10 g/d (4 g urea nitrogen excreted/d + 2.17 g NUN/d + 4.2 g urea nitrogen accumulated/d = 10.3 g nitrogen/d). • Because his nitrogen excretion substantially exceeds the dietary nitrogen of 6.4 g/d, he requires a consultation with a nutrition/dietician and testing for gastrointestinal bleeding
SUN, serum urea nitrogen; NUN, nonurea nitrogen excretion.

reactive oxygen species initiates similar events in patients with CKD.

Another potential role of urea in producing uremia-induced toxicity is through the development of protein carbamylation, which could disrupt the structure of a protein interfering with signaling pathways and so forth. Stim *et al.* reported that the rate of carbamylation of hemoglobin increased in parallel with the increase in SUN and that carbamylation was significantly higher in patients with ESRD compared with normal individuals (99). These responses were confirmed by Berg *et al.* (100) except that the carbamylated protein was albumin, rather than hemoglobin. Thus, carbamylation of several proteins can occur in uremic individuals but whether this produces toxic reactions has not been defined.

Finally, there are patient-based reports that cast doubt on the hypothesis that urea is a toxin. Hsu *et al.* studied a man and a woman from a family of patients who had chronic but unexplained azotemia. Results of the evaluation indicated that the high SUN arose from a autosomal dominant genetic defect in urea reabsorption (101). Kidney function of the two participants revealed subnormal urea clearances but otherwise normal values of inulin clearance, urea excretion, and responses of urea clearance to diuresis and antidiuresis plus normal sodium clearances. Although the mechanism for the familial azotemia was not identified, the report is relevant because the participants had no clinical or laboratory findings attributable to the increase in SUN despite years of values varying from 49 to 65 mg/dl and from 55 to 60 mg/dl, respectively. In another case study, Richards and Brown studied a woman with prolonged azotemia to examine the association between a high SUN and the development of uremic symptoms (102). The participant subsisted on a diet consisting primarily of fish and a protein powder, yielding urea nitrogen production rates of 40–50 g/d for years. Although the participant maintained a SUN of 50–80 mg/dl for years, she had normal values of hemoglobin, plasma creatinine, BP, and no weight loss. Together, these reports indicate that even a prolonged increase in the concentration of urea does not produce toxic reactions, at least in patients with normal kidney function.

Urea is the largest circulating pool of nitrogen and its production changes in parallel to the degradation of dietary and endogenous proteins. These facts and other properties of urea can be used to estimate the degree of uremia and the compliance with prescribed amounts protein in the diet. The available evidence in patients with CKD suggests that reutilization of ammonia derived from urea degradation for the synthesis of amino acids and proteins is minimal. Whether the evidence would be more persuasive under extreme conditions, such as in hibernating bears, is unknown. The ability of urea to create toxicity is unsettled but years of high SUN values do not produce toxic reactions in individuals with otherwise normal kidney function.

In conclusion, renal urea and ammonia metabolism mediate critical roles in nitrogen balance, urine concentration, and acid-base homeostasis. In this review, we evaluated critical processes involved in these homeostatic mechanisms. Abnormal urea and ammonia metabolism both result from and can lead to a wide variety of conditions, including methods for evaluating issues that are critical to caring for patients with impaired renal function.

Acknowledgments

The preparation of this review was supported by funds from the National Institutes of Health (R37-DK037175 to W.E.M., R01-DK045788 to I.D.W., and R01-DK089828 and R21-DK091147 to J.M.S.) and the US Department of Veterans Affairs (1101BX000818 to I.D.W.).

Disclosures

None.

References

- Mitch WE, Goldberg AL: Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *N Engl J Med* 335: 1897–1905, 1996
- Lecker SH, Mitch WE: Proteolysis by the ubiquitin-proteasome system and kidney disease. *J Am Soc Nephrol* 22: 821–824, 2011
- Franch HA, Mitch WE: Navigating between the Scylla and Charybdis of prescribing dietary protein for chronic kidney diseases. *Annu Rev Nutr* 29: 341–364, 2009
- Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27: 58–65, 1985
- Addis T: The ratio between the urea content of the urine and of the blood after the administration of large quantities of urea: An approximate index of the quantity of actively functioning kidney tissue. *J Urol* 1: 263–287, 1917
- Addis T, Lippman RW, Lew W, Poo LJ, Wong W: Effect of dietary protein consumption upon body growth and organ size in the rat. *Am J Physiol* 165: 491–496, 1951
- Sitpriya V, Suvanpha R: Low protein diet and chronic renal failure in Buddhist monks. *Br Med J (Clin Res Ed)* 287: 469–471, 1983
- Hostetter TH: Human renal response to meat meal. *Am J Physiol* 250: F613–F618, 1986
- Moore LW, Byham-Gray LD, Scott Parrott J, Rigassio-Radler D, Mandayam S, Jones SL, Mitch WE, Osama Gaber A: The mean dietary protein intake at different stages of chronic kidney disease is higher than current guidelines. *Kidney Int* 83: 724–732, 2013
- Bagnasco SM, Peng T, Janech MG, Karakashian A, Sands JM: Cloning and characterization of the human urea transporter UT-A1 and mapping of the human Slc14a2 gene. *Am J Physiol Renal Physiol* 281: F400–F406, 2001
- Klein JD, Blount MA, Sands JM: Urea transport in the kidney. *Compr Physiol* 1: 699–729, 2011
- Klein JD, Blount MA, Sands JM: Molecular mechanisms of urea transport in health and disease. *Pflügers Arch* 464: 561–572, 2012
- Blount MA, Klein JD, Sands JM: UT (Urea Transporter). In: *Encyclopedia of Signaling Molecules*, edited by Choi S, New York, Springer, 2012, pp 1945–1953
- Blount MA, Klein JD, Martin CF, Tchapyjnikov D, Sands JM: Forskolin stimulates phosphorylation and membrane accumulation of UT-A3. *Am J Physiol Renal Physiol* 293: F1308–F1313, 2007
- Terris JM, Knepper MA, Wade JB: UT-A3: Localization and characterization of an additional urea transporter isoform in the IMCD. *Am J Physiol Renal Physiol* 280: F325–F332, 2001
- Sands JM, Layton HE: The urine concentrating mechanism and urea transporters. In: *Seldin and Giebisch's The Kidney: Physiology and Pathophysiology*, edited by Alpern RJ, Caplan MJ, Moe OW, San Diego, Academic Press, 2013, pp 1463–1510
- Sands JM, Layton HE, Fenton RA: Urine concentration and dilution. In: *Brenner and Rector's The Kidney*, edited by Taal M, Chertow GM, Marsden PA, Skorecki K, Yu A, Brenner BM, Philadelphia, Elsevier, 2011, pp 326–352
- Pannabecker TL, Dantzer WH, Layton HE, Layton AT: Role of three-dimensional architecture in the urine concentrating mechanism of the rat renal inner medulla. *Am J Physiol Renal Physiol* 295: F1271–F1285, 2008
- Gamble JL, McKhann CF, Butler AM, Tuthill E: An economy of water in renal function referable to urea. *Am J Physiol* 109: 139–154, 1934

20. Kokko JP, Rector FC Jr: Countercurrent multiplication system without active transport in inner medulla. *Kidney Int* 2: 214–223, 1972
21. Stephenson JL: Concentration of urine in a central core model of the renal counterflow system. *Kidney Int* 2: 85–94, 1972
22. Sands JM, Knepper MA: Urea permeability of mammalian inner medullary collecting duct system and papillary surface epithelium. *J Clin Invest* 79: 138–147, 1987
23. Sands JM, Gargus JJ, Fröhlich O, Gunn RB, Kokko JP: Urinary concentrating ability in patients with Jk(a-b-) blood type who lack carrier-mediated urea transport. *J Am Soc Nephrol* 2: 1689–1696, 1992
24. Yang B, Bankir L, Gillespie A, Epstein CJ, Verkman AS: Urea-selective concentrating defect in transgenic mice lacking urea transporter UT-B. *J Biol Chem* 277: 10633–10637, 2002
25. Macey RI: Transport of water and urea in red blood cells. *Am J Physiol* 246: C195–C203, 1984
26. Zhang C, Sands JM, Klein JD: Vasopressin rapidly increases phosphorylation of UT-A1 urea transporter in rat IMCDs through PKA. *Am J Physiol Renal Physiol* 282: F85–F90, 2002
27. Blount MA, Mistry AC, Fröhlich O, Price SR, Chen G, Sands JM, Klein JD: Phosphorylation of UT-A1 urea transporter at serines 486 and 499 is important for vasopressin-regulated activity and membrane accumulation. *Am J Physiol Renal Physiol* 295: F295–F299, 2008
28. Wang Y, Klein JD, Blount MA, Martin CF, Kent KJ, Pech V, Wall SM, Sands JM: Epac regulates UT-A1 to increase urea transport in inner medullary collecting ducts. *J Am Soc Nephrol* 20: 2018–2024, 2009
29. Fenton RA, Chou CL, Stewart GS, Smith CP, Knepper MA: Urinary concentrating defect in mice with selective deletion of phloretin-sensitive urea transporters in the renal collecting duct. *Proc Natl Acad Sci U S A* 101: 7469–7474, 2004
30. Sands JM, Schrader DC: An independent effect of osmolality on urea transport in rat terminal inner medullary collecting ducts. *J Clin Invest* 88: 137–142, 1991
31. Wang Y, Klein JD, Fröhlich O, Sands JM: Role of protein kinase C- α in hypertonicity-stimulated urea permeability in mouse inner medullary collecting ducts. *Am J Physiol Renal Physiol* 304: F233–F238, 2013
32. Blessing NW, Blount MA, Sands JM, Martin CF, Klein JD: Urea transporters UT-A1 and UT-A3 accumulate in the plasma membrane in response to increased hypertonicity. *Am J Physiol Renal Physiol* 295: F1336–F1341, 2008
33. Klein JD, Fröhlich O, Blount MA, Martin CF, Smith TD, Sands JM: Vasopressin increases plasma membrane accumulation of urea transporter UT-A1 in rat inner medullary collecting ducts. *J Am Soc Nephrol* 17: 2680–2686, 2006
34. Klein JD, Martin CF, Kent KJ, Sands JM: Protein kinase C- α mediates hypertonicity-stimulated increase in urea transporter phosphorylation in the inner medullary collecting duct. *Am J Physiol Renal Physiol* 302: F1098–F1103, 2012
35. Yao L, Huang DY, Pfaff IL, Nie X, Leitges M, Vallon V: Evidence for a role of protein kinase C- α in urine concentration. *Am J Physiol Renal Physiol* 287: F299–F304, 2004
36. Kim D, Sands JM, Klein JD: Role of vasopressin in diabetes mellitus-induced changes in medullary transport proteins involved in urine concentration in Brattleboro rats. *Am J Physiol Renal Physiol* 286: F760–F766, 2004
37. Peil AE, Stolte H, Schmidt-Nielsen B: Uncoupling of glomerular and tubular regulations of urea excretion in rat. *Am J Physiol* 258: F1666–F1674, 1990
38. Knepper MA, Danielson RA, Saidel GM, Johnston KH: Effects of dietary protein restriction and glucocorticoid administration on urea excretion in rats. *Kidney Int* 8: 303–315, 1975
39. Naruse M, Klein JD, Ashkar ZM, Jacobs JD, Sands JM: Glucocorticoids downregulate the vasopressin-regulated urea transporter in rat terminal inner medullary collecting ducts. *J Am Soc Nephrol* 8: 517–523, 1997
40. Li C, Wang W, Summer SN, Falk S, Schrier RW: Downregulation of UT-A1/UT-A3 is associated with urinary concentrating defect in glucocorticoid-excess state. *J Am Soc Nephrol* 19: 1975–1981, 2008
41. Gertner RA, Klein JD, Bailey JL, Kim DU, Luo XH, Bagnasco SM, Sands JM: Aldosterone decreases UT-A1 urea transporter expression via the mineralocorticoid receptor. *J Am Soc Nephrol* 15: 558–565, 2004
42. Bailey JL, Mitch WE: Twice-told tales of metabolic acidosis, glucocorticoids, and protein wasting: What do results from rats tell us about patients with kidney disease? *Semin Dial* 13: 227–231, 2000
43. Klein JD, Rouillard P, Roberts BR, Sands JM: Acidosis mediates the upregulation of UT-A protein in livers from uremic rats. *J Am Soc Nephrol* 13: 581–587, 2002
44. Jung JY, Madsen KM, Han KH, Yang CW, Knepper MA, Sands JM, Kim J: Expression of urea transporters in potassium-depleted mouse kidney. *Am J Physiol Renal Physiol* 285: F1210–F1224, 2003
45. Jeon US, Han KH, Park SH, Lee SD, Sheen MR, Jung JY, Kim WY, Sands JM, Kim J, Kwon HM: Downregulation of renal TonEBP in hypokalemic rats. *Am J Physiol Renal Physiol* 293: F408–F415, 2007
46. Elkinton JR, Huth EJ, Webster GD Jr, McCance RA: The renal excretion of hydrogen ion in renal tubular acidosis. I. quantitative assessment of the response to ammonium chloride as an acid load. *Am J Med* 29: 554–575, 1960
47. Weiner ID, Verlander JW: Renal ammonia metabolism and transport. *Compr Physiol* 3: 201–220, 2013
48. Van Slyke DD, Phillips RA, Hamilton PB, Archibard RM, Fletcher PH, Miller A: Glutamine as source material of urinary ammonia. *J Biol Chem* 150: 481–482, 1943
49. Solbu TT, Boulland JL, Zahid W, Lyamouri Bredahl MK, Amiry-Moghaddam M, Storm-Mathisen J, Roberg BA, Chaudhry FA: Induction and targeting of the glutamine transporter SN1 to the basolateral membranes of cortical kidney tubule cells during chronic metabolic acidosis suggest a role in pH regulation. *J Am Soc Nephrol* 16: 869–877, 2005
50. Moret C, Dave MH, Schulz N, Jiang JX, Verrey F, Wagner CA: Regulation of renal amino acid transporters during metabolic acidosis. *Am J Physiol Renal Physiol* 292: F555–F566, 2007
51. Curthoys NP, Moe OW: Proximal tubule function and response to acidosis [published online ahead of print May 1, 2014]. *Clin J Am Soc Nephrol* doi:10.2215/CJN.10391012
52. Tannen RL: Ammonia and acid-base homeostasis. *Med Clin North Am* 67: 781–798, 1983
53. Weiner ID, Hamm LL: Molecular mechanisms of renal ammonia transport. *Annu Rev Physiol* 69: 317–340, 2007
54. Nagami GT: Ammonia production and secretion by isolated perfused proximal tubule segments. *Miner Electrolyte Metab* 16: 259–263, 1990
55. Hamm LL, Simon EE: Roles and mechanisms of urinary buffer excretion. *Am J Physiol* 253: F595–F605, 1987
56. Bourgeois S, Meer LV, Wootla B, Bloch-Faure M, Chambrey R, Shull GE, Gawenis LR, Houillier P: NHE4 is critical for the renal handling of ammonia in rodents. *J Clin Invest* 120: 1895–1904, 2010
57. Kikeri D, Sun A, Zeidel ML, Hebert SC: Cell membranes impermeable to NH₃. *Nature* 339: 478–480, 1989
58. Lee S, Lee HJ, Yang HS, Thornell IM, Benveniste MO, Choi I: Sodium-bicarbonate cotransporter NBCn1 in the kidney medullary thick ascending limb cell line is upregulated under acidic conditions and enhances ammonium transport. *Exp Physiol* 95: 926–937, 2010
59. DuBose TD Jr, Good DW, Hamm LL, Wall SM: Ammonium transport in the kidney: New physiological concepts and their clinical implications. *J Am Soc Nephrol* 1: 1193–1203, 1991
60. Biver S, Belge H, Bourgeois S, Van Vooren P, Nowik M, Scohy S, Houillier P, Szpirer J, Szpirer C, Wagner CA, Devuyst O, Marini AM: A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility. *Nature* 456: 339–343, 2008
61. Bishop JM, Verlander JW, Lee HW, Nelson RD, Weiner AJ, Handlogten ME, Weiner ID: Role of the Rhesus glycoprotein, Rh B glycoprotein, in renal ammonia excretion. *Am J Physiol Renal Physiol* 299: F1065–F1077, 2010
62. Lee HW, Verlander JW, Bishop JM, Igarashi P, Handlogten ME, Weiner ID: Collecting duct-specific Rh C glycoprotein deletion alters basal and acidosis-stimulated renal ammonia excretion. *Am J Physiol Renal Physiol* 296: F1364–F1375, 2009
63. Wall SM: Ammonium transport and the role of the Na,K-ATPase. *Miner Electrolyte Metab* 22: 311–317, 1996

64. Stettner P, Bourgeois S, Marsching C, Traykova-Brauch M, Porubsky S, Nordström V, Hopf C, Koesters R, Sandhoff R, Wiegandt H, Wagner CA, Gröne HJ, Jennemann R: Sulfatides are required for renal adaptation to chronic metabolic acidosis. *Proc Natl Acad Sci U S A* 110: 9998–10003, 2013
65. May RC, Kelly RA, Mitch WE: Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. *J Clin Invest* 77: 614–621, 1986
66. de Brito-Ashurst I, Varagunam M, Raftery MJ, Yaqoob MM: Bicarbonate supplementation slows progression of CKD and improves nutritional status. *J Am Soc Nephrol* 20: 2075–2084, 2009
67. Abramowitz MK, Melamed ML, Bauer C, Raff AC, Hostetter TH: Effects of oral sodium bicarbonate in patients with CKD. *Clin J Am Soc Nephrol* 8: 714–720, 2013
68. Knochel JP: Diuretic-induced hypokalemia. *Am J Med* 77: 18–27, 1984
69. Hulter HN, Ilnicki LP, Harbottle JA, Sebastian A: Impaired renal H⁺ secretion and NH₃ production in mineralocorticoid-deficient glucocorticoid-replete dogs. *Am J Physiol* 232: F136–F146, 1977
70. Welbourne TC, Givens G, Joshi S: Renal ammoniagenic response to chronic acid loading: Role of glucocorticoids. *Am J Physiol* 254: F134–F138, 1988
71. Karinch AM, Lin CM, Meng Q, Pan M, Souba WW: Glucocorticoids have a role in renal cortical expression of the SNAT3 glutamine transporter during chronic metabolic acidosis. *Am J Physiol Renal Physiol* 292: F448–F455, 2007
72. Cassuto H, Olswang Y, Heinemann S, Sabbagh K, Hanson RW, Reshef L: The transcriptional regulation of phosphoenolpyruvate carboxykinase gene in the kidney requires the HNF-1 binding site of the gene. *Gene* 318: 177–184, 2003
73. Wang D, Sun H, Lang F, Yun CC: Activation of NHE3 by dexamethasone requires phosphorylation of NHE3 at Ser663 by SGK1. *Am J Physiol Cell Physiol* 289: C802–C810, 2005
74. Bobulescu IA, Dwarakanath V, Zou L, Zhang J, Baum M, Moe OW: Glucocorticoids acutely increase cell surface Na⁺/H⁺ exchanger-3 (NHE3) by activation of NHE3 exocytosis. *Am J Physiol Renal Physiol* 289: F685–F691, 2005
75. Remer T, Manz F: Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am J Clin Nutr* 59: 1356–1361, 1994
76. Busque SM, Wagner CA: Potassium restriction, high protein intake, and metabolic acidosis increase expression of the glutamine transporter SNAT3 (Slc38a3) in mouse kidney. *Am J Physiol Renal Physiol* 297: F440–F450, 2009
77. Welters CF, Deutz NE, Dejong CH, Soeters PB: Enhanced renal vein ammonia efflux after a protein meal in the pig. *J Hepatol* 31: 489–496, 1999
78. Olde Damink SW, Jalan R, Deutz NE, Redhead DN, Dejong CH, Hynd P, Jalan RA, Hayes PC, Soeters PB: The kidney plays a major role in the hyperammonemia seen after simulated or actual GI bleeding in patients with cirrhosis. *Hepatology* 37: 1277–1285, 2003
79. Mitch WE, Fouque D: Dietary approaches to kidney disease. In: *Brenner and Rector's The Kidney*, edited by Brenner BM, Philadelphia, Elsevier, 2012, pp 2170–2204
80. Beale LS: *Kidney Diseases, Urinary Deposits and Calculous Disorders; Their Nature and Treatment*, Philadelphia, Lindsay and Blakiston, 1869
81. Folin O: Laws governing the clinical composition of urine. *Am J Physiol* 13: 67–115, 1905
82. Cottini EP, Gallina DL, Dominguez JM: Urea excretion in adult humans with varying degrees of kidney malfunction fed milk, egg or an amino acid mixture: Assessment of nitrogen balance. *J Nutr* 103: 11–19, 1973
83. Mitch WE, Remuzzi G: Diets for patients with chronic kidney disease, still worth prescribing. *J Am Soc Nephrol* 15: 234–237, 2004
84. Vegter S, Perna A, Postma MJ, Navis G, Remuzzi G, Ruggenenti P: Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol* 23: 165–173, 2012
85. Zoccali C, Ruggenenti P, Perna A, Leonardis D, Tripepi R, Tripepi G, Mallamaci F, Remuzzi G; REIN Study Group: Phosphate may promote CKD progression and attenuate renoprotective effect of ACE inhibition. *J Am Soc Nephrol* 22: 1923–1930, 2011
86. Rafoth RJ, Onstad GR: Urea synthesis after oral protein ingestion in man. *J Clin Invest* 56: 1170–1174, 1975
87. Mitch WE, Wilcox CS: Disorders of body fluids, sodium and potassium in chronic renal failure. *Am J Med* 72: 536–550, 1982
88. Walser M: Determinants of ureagenesis, with particular reference to renal failure. *Kidney Int* 17: 709–721, 1980
89. Masud T, Manatunga A, Cotsonis G, Mitch WE: The precision of estimating protein intake of patients with chronic renal failure. *Kidney Int* 62: 1750–1756, 2002
90. Mitch WE, Lietman PS, Walser M: Effects of oral neomycin and kanamycin in chronic uremic patients: I. Urea metabolism. *Kidney Int* 11: 116–122, 1977
91. Richards P, Metcalfe-Gibson A, Ward EE, Wrong O, Houghton BJ: Utilisation of ammonia nitrogen for protein synthesis in man, and the effect of protein restriction and uraemia. *Lancet* 2: 845–849, 1967
92. Mitch WE, Walser M: Effects of oral neomycin and kanamycin in chronic uremic patients: II. Nitrogen balance. *Kidney Int* 11: 123–127, 1977
93. Varcoe R, Halliday D, Carson ER, Richards P, Tavill AS: Efficiency of utilization of urea nitrogen for albumin synthesis by chronically uraemic and normal man. *Clin Sci Mol Med* 48: 379–390, 1975
94. Stenvinkel P, Jani AH, Johnson RJ: Hibernating bears (Ursidae): Metabolic magicians of definite interest for the nephrologist. *Kidney Int* 83: 207–212, 2013
95. Johnson WJ, Hagge WW, Waggoner RD, Dinapoli RP, Rosevear JW: Effects of urea loading in patients with far-advanced renal failure. *Mayo Clin Proc* 47: 21–29, 1972
96. Reddy ST, Wang CY, Sakhaee K, Brinkley L, Pak CY: Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism. *Am J Kidney Dis* 40: 265–274, 2002
97. Thomas SS, Dong Y, Zhang L, Mitch WE: Signal regulatory protein- α interacts with the insulin receptor contributing to muscle wasting in chronic kidney disease. *Kidney Int* 84: 308–316, 2013
98. Zhang L, Pan J, Dong Y, Twardy DJ, Dong Y, Garibotto G, Mitch WE: Stat3 activation links a C/EBP δ to myostatin pathway to stimulate loss of muscle mass. *Cell Metab* 18: 368–379, 2013
99. Stim J, Shaykh M, Anwar F, Ansari A, Arruda JAL, Dunea G: Factors determining hemoglobin carbamylation in renal failure. *Kidney Int* 48: 1605–1610, 1995
100. Berg AH, Drechsler C, Wenger J, Bucsfusca R, Hod T, Kalim S, Ramma W, Parikh SM, Steen H, Friedman DJ, Danziger J, Wanner C, Thadhani R, Karumanchi SA: Carbamylation of serum albumin as a risk factor for mortality in patients with kidney failure. *Sci Transl Med* 5: 175ra29, 2013
101. Hsu CH, Kurtz TW, Massari PU, Ponce SA, Chang BS: Familial azotemia. Impaired urea excretion despite normal renal function. *N Engl J Med* 298: 117–121, 1978
102. Richards P, Brown CL: Urea metabolism in an azotaemic woman with normal renal function. *Lancet* 2: 207–209, 1975



Chemical and Physical Sensors in the Regulation of Renal Function

Jennifer L. Pluznick* and Michael J. Caplan†

Abstract

In order to assess the status of the volume and composition of the body fluid compartment, the kidney monitors a wide variety of chemical and physical parameters. It has recently become clear that the kidney's sensory capacity extends well beyond its ability to sense ion concentrations in the forming urine. The kidney also keeps track of organic metabolites derived from a surprising variety of sources and uses a complex interplay of physical and chemical sensing mechanisms to measure the rate of fluid flow in the nephron. Recent research has provided new insights into the nature of these sensory mechanisms and their relevance to renal function.

Clin J Am Soc Nephrol 10: 1626–1635, 2015. doi: 10.2215/CJN.00730114

*Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland; and †Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut

Introduction

It has long been appreciated that we use sensory receptors to detect the chemical and physical properties of the world around us. However, it has recently become clear that we also utilize the same molecular tools to monitor the composition of the world within us. Many sensory receptors, including olfactory receptors (ORs), taste receptors, and other sensory G protein-coupled receptors (GPCRs), as well as receptors that function as mechanosensitive or chemosensitive ion channels, have recently been shown to play key roles in organs and tissues traditionally thought of as “nonsensory.” In the past few years, a large and diverse literature has developed documenting systems in which “sensory” receptors are exploited to serve in a wide variety of physiologic processes. The tongue's sour taste receptors, for example, are also called upon to sense pH in the spinal column (1), its bitter taste receptors also regulate bronchodilation and ciliary beat frequency in the lung in response to certain inhalants (2,3), and its sweet taste receptors regulate glucose transport in the gut (4,5). In addition to taste receptors, there are also numerous examples of ORs playing a variety of roles in tissues outside of the nose. The OR gene family, in fact, is the largest gene family in the genome (6–8), and thus forms an expansive repertoire of GPCR-based chemical detectors. In addition to odorant detection in the nose, ORs are now understood to also mediate chemical detection in other environments. For example, ORs play roles in muscle cell migration and sperm chemotaxis (9). Ligands for these receptors are often produced as a result of metabolic processes, implying that seemingly inert intermediate metabolites or by-products may have unappreciated signaling roles (1,10–13).

The kidney stands out as a particularly appropriate tissue in which to deploy sensory receptors for chemodetection. The kidney evaluates and maintains a large number of physiologic parameters, including systemic acid-base balance, electrolyte concentrations, volume

status, and toxin levels. To achieve this, the kidney must monitor the concentration of numerous substances in the plasma and the early urine. Sensory chemoreceptors are by their nature well suited to play central roles in these renal “chemosensory” tasks.

In addition to sensing chemical cues, the kidney must also monitor physical or mechanical stimuli, such as shear stress, pressure, and flow rate. Recent studies have shown that fluid flow is an important sensory cue in the acute regulation of urine composition (14) and in the proper development and maintenance of the structure of the nephron. Studies in the fruit fly have shown that mechanical stimulation is important in regulating the pathways that control planar cell polarity (15), which is the process through which neighboring epithelial cells acquire distinct structural and functional properties. Planar cell polarity signaling pathways are critically important in the differentiation of the distinct epithelial cell types that constitute the nephron. Impairments in fluid flow detection may play roles in the development of clinically significant diseases of the kidney (16,17). Mechanical forces are important regulators of the activity of renal ion channels (18–25), as well as of the signaling activities that regulate renal ion and fluid transport (14,26–28). Therefore, the capacity to monitor mechanical sensations is exploited by the kidney in its efforts to maintain homeostasis. In this review, we discuss recent studies that reveal surprising and important roles for mechanosensitive and chemosensory molecular machinery in renal function.

Physiology and Pathophysiology of Renal Cilia

With the possible exception of the intercalated cells of the collecting duct (29), every epithelial cell that lines the nephron is endowed with a single primary cilium (30). These cilia arise from the apical surfaces of the epithelial cells and protrude into the tubule lumen. The typical renal epithelial cell cilium is 2- to 3- μ m

Correspondence:

Dr. Michael J. Caplan, Department of Cellular and Molecular Physiology, Yale University School of Medicine, PO Box 208026, New Haven, CT 06520-8026. Email: michael.caplan@yale.edu

long and $<1\ \mu\text{m}$ in cross-sectional width. The cilium's surface membrane is continuous with that of the apical plasma membrane, although its lipid and protein compositions differ substantially from those of the surrounding apical cell surface (31). Just beneath the ciliary membrane is the ciliary axoneme, which is a scaffolding composed of nine doublet microtubules that are parallel to one another and that extend from the base of the cilium along its entire axial length. In contrast with the motile cilia that are found on airway and oviduct epithelial cells, primary cilia, such as those found on renal epithelial cells, do not beat and do not move fluid. Instead, their function appears to be entirely sensory and their structure reflects their sedentary nature. The axonemes of motile cilia possess a central pair of microtubules that is connected by radial spokes to the nine doublet microtubules at the ciliary periphery. These additional components include the motor proteins that allow motile cilia to generate force. Although primary cilia, such as those found in the kidney, lack these components found in their motile cousins, they are by no means devoid of motor proteins. Large protein assemblies known as intraflagellar transport (IFT) complexes exploit motor proteins to crawl up and down the axonemal microtubules. These IFT complexes serve as delivery machinery that transport newly synthesized proteins into the cilium and carry away components that are destined for removal (32,33). The IFT complexes are absolutely required in order for primary cilia to form and to function. It should also be noted that every cilium arises from a basal body, which is a structure composed of paired cylindrical assemblies of microtubules. The basal body serves as the centriole that organizes the mitotic spindle in dividing cells. A complex array of proteins connects the base of the axoneme to the basal body and forms the ciliary transition zone, which serves as a barrier that helps to maintain the compositional distinctions between the apical and ciliary membrane domains (34,35).

Although the structure of the renal primary cilium is fairly well understood, the same cannot be said of its function. It is clear that the cilia of renal epithelial cells serve extremely important purposes, as evidenced by the large number of renal phenotypes arising from mutations in genes whose products participate in ciliogenesis (36). Bardet-Biedl syndrome, nephronophthisis, Meckel-Gruber syndrome, Joubert's syndrome, Senior-Løken syndrome, and orofacioidigital syndrome are all caused by loss-of-function mutations in genes that encode distinct subclasses of the components of the cellular machinery that is required to build and maintain the primary cilium (37,38). These pleomorphic conditions are characterized by partially overlapping lists of neurologic, skeletal, metabolic, and sensory phenotypes, including renal cystic disease. In addition, the proteins encoded by the genes responsible for both the autosomal dominant and autosomal recessive forms of polycystic kidney disease localize, at least in part, to the primary cilium (39–41). On the basis of this brief summary, it might be logical to suggest that the cilium participates in sending signals that are required to prevent the development of renal cysts. Recent data, however, suggest that the cilium's role in renal cystic disease, although critical, is not this straightforward.

The groundbreaking work of Praetorius and Spring revealed that the primary cilium can detect and respond

to mechanical stimuli (42). These investigators showed that either direct or flow-induced bending of the primary cilia of cultured renal epithelial cells led to the activation of ion channels that mediated calcium influx, which secondarily activated calcium release from intracellular stores. Studies into the function of the proteins encoded by the autosomal dominant polycystic kidney disease genes (41) have provided insight into the nature of the mechanosensitive ion channels responsible for this cilia-dependent activity. Polycystin-1, the product of the *Pkd1* gene, encodes a massive protein. It is composed of 4302 amino acids and spans the membrane 11 times. Polycystin-2, encoded by the *Pkd2* gene, is roughly one fourth of the size of polycystin-1 and spans the membrane 6 times. Polycystin-2 belongs to the transient receptor potential (TRP) family of calcium-permeable ion channels. Polycystin-1 and polycystin-2 interact with one another to form a complex that localizes in part to the cilium and that may contribute to the calcium channel activity that is induced by ciliary bending. This activity may depend upon TrpV4, another member of the Trp family of cation channels whose channel activity may be regulated in some manner by the polycystin proteins (43). These observations have inspired a model in which the ciliary population of the polycystin-1 and polycystin-2 complex serves as a sensor that transduces tubular fluid flow to produce an elevation of renal epithelial cell cytoplasmic calcium concentrations (44). These observations prompt the further suggestion that loss of this mechanically activated polycystin channel activity, or of the mechanosensitive cilium in which this activity resides, could lead to the perturbations in cell proliferation, differentiation, and fluid secretion that together characterize the formation of autosomal dominant polycystic kidney disease renal cysts. Recent data indicate that close relatives of polycystins, rather than the polycystin-1 and polycystin-2, may mediate ciliary ion currents in at least some cell types (45,46).

Although the role of the ciliary polycystins as flow sensors is intriguing, it seems quite likely that these proteins also participate in other sensory processes. In addition to their connection to cytoplasmic calcium levels, the polycystin proteins have been connected to a very large and diverse collection of signaling pathways that have the potential to influence cellular growth and metabolism (41). Furthermore, polycystin-1 interacts with trimeric G proteins, suggesting the intriguing possibility that it functions as an atypical GPCR (47). Finally, it is worth noting that both polycystin proteins have homologs that have been shown to serve as chemosensors. Polycystin-1-like-3 and polycystin-2-like-1 form a complex that detects low pH, serving both as sour taste receptors in the tongue and as sensors of pH in the central nervous system (1,48,49). Taken together, these facts suggest that the polycystins may serve chemosensory roles in renal epithelial cells. The nature of the ligands to which the polycystins might respond and the subcellular localization in which the polycystins perform this putative activity remain to be determined.

As noted above, the connection between ciliary function and the prevention of renal cystic disease has recently become more complex. Clearly, the pathology associated with the ciliopathies confirms that loss of cilia is sufficient to lead to the development of renal cysts. Surprisingly, however, studies utilizing mouse models reveal that loss of cilia can suppress cyst formation that is caused by the loss

of polycystin-1 or polycystin-2 expression (50). This unexpected observation suggests that, in the absence of the polycystin proteins, cilia send a message or messages that activate cystogenic processes. The polycystins appear to counteract or suppress these messages. The nature of these putative procystogenic and countercystogenic messages remains to be determined. Elucidating the molecular details of this apparently antagonistic relationship between the cilium and the polycystins will no doubt provide interesting insights into the mechanisms through which renal epithelial cells exploit cilia and the polycystins to interrogate the chemical composition and flow rate of the tubular fluid.

Although flow-induced ciliary bending may activate ion channels directly, it is clear that this mechanical stimulus also initiates a fascinating autocrine chemical signaling process (Figure 1). Bending of the primary cilium causes renal epithelial cells to release ATP into the tubule lumen (51). This ATP appears to be stored inside the cell in vesicular compartments that, upon stimulation, fuse with the apical plasma membrane and release their contents (52). This extracellular ATP can bind to and activate purinergic receptors that are present on the apical plasma membranes of renal epithelial cells. There are two distinct families of purinergic receptors, each of which has many branches. Members of the P2Y family are metabotropic receptors that signal through trimeric G proteins, whereas the P2X receptors are ligand-gated ion channels (53). Both types of purinergic receptors populate the apical and basolateral surfaces of renal epithelial cells. Thus, flow-induced ATP

release can activate both ionic currents and intracellular second messenger pathways. Each nephron segment expresses a distinct collection of purinergic receptors, distributed among their apical and basolateral plasma membrane domains, which modulate the tubule segment's physiologic properties in response to the various physiologic stimuli that are transmitted through elevations in extracellular nucleotide concentrations (54). Activation of the apical P2Y G protein-coupled purinergic receptor in the renal collecting duct, for example, results in activation of protein kinase C, which, in turn, inhibits the epithelial sodium channel (ENaC) and, consequently, reduces transepithelial sodium reabsorption (55). Thus, in this segment, high flow rates can be sensed by the bending of the primary cilium and communicated to the epithelial ion transport machinery through the release of ATP *via* fusion of intracellular vesicles with the apical plasma membrane. This autocrine and paracrine messenger acts through purinergic receptors to reduce sodium uptake. Hence, high flow rates, which can arise as a consequence of expansion of the extracellular fluid volume leading to an increase in the GFR, reduce the retention of sodium and volume. Through a collaboration between mechanical and chemical sensory tools, this multicomponent and multimodal mechanism thus underlies a logical and elegant physiologic adaptation.

The intriguing mechanism described in the preceding paragraph by no means constitutes the only example of renal tubule fluid flow being sensed or interpreted to influence the composition of the forming urine. The process

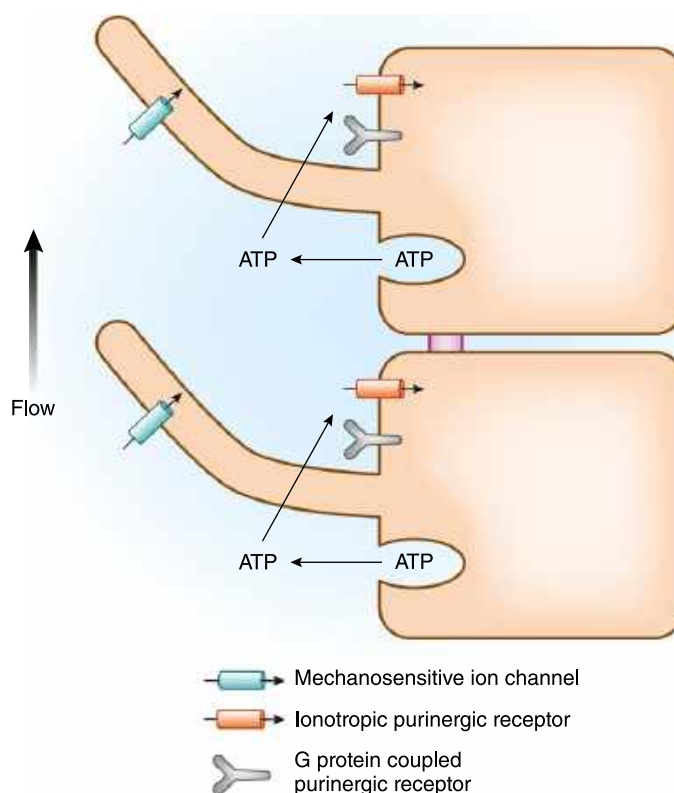


Figure 1. | Flow-induced bending of the primary cilium can be transduced through several mechanisms, including the activation of mechanosensory ion channels and the release of vesicular ATP, which, in turn, activates purinergic receptors.

of tubuloglomerular feedback is a classic example of flow rates being “sensed” (as an index of changes in tubular fluid electrolyte concentrations) to mediate changes in renal function. In addition, the large-conductance Ca^{2+} -sensitive potassium channel of the renal collecting duct is responsible for the capacity of the late nephron to increase potassium secretion in response to increased flow rates (25,56). Therefore, a variety of renal mechanisms depend upon a complex combination of sensors and signaling pathways that participate in adjusting the kidney’s capacity to modify the forming urine to the rate at which it is flowing through the tubule lumen.

Finally, it is worth noting that cilia are not the only mechanosensitive cellular organelles that extend into the lumen of the renal tubule. The microvilli that constitute the luxuriant brush borders of proximal tubule epithelial cells may also manifest the capacity to monitor and communicate tubular fluid flow rates (14). Flow-induced microvillar bending, communicated through elements of the cytoskeleton to the intracellular signaling machinery that regulates transport processes, may play an important role in governing transepithelial uptake of sodium, bicarbonate, and water in the initial segment of the nephron (57).

Renal Chemosensors

The kidney monitors and responds to the levels of a number of key metabolites. In this review, we focus on the chemosensors that detect succinate, short chain fatty acids (SCFAs), and bicarbonate.

Succinate

Succinate, which participates in the tricarboxylic acid (TCA; or citric acid) cycle, resides primarily in the mitochondria. However, this metabolite is also found in the plasma in the

micromolar range (11), where it plays an important signaling role. It has been suggested that plasma succinate levels are an index of the level of hypoxia and/or of oxidative or mitochondrial stress, based in part on the observation that tissue levels of succinate rise in hypoxic conditions while the levels of other TCA cycle intermediates fall (58,59). Succinate administration increases BP in a dose-dependent manner (11), and the succinate receptor GPCR 91 (Gpr91) was originally identified as a mediator of the hypertensive response to succinate (11,60). The initial characterization of Gpr91 clearly demonstrated that this receptor is highly expressed in the kidney and that the hypertensive response to succinate was dependent on the renin-angiotensin system (RAS) (11). The details of the mechanism underlying this phenomenon were unraveled in several studies that revealed that Gpr91 is localized in the distal nephron, including the macula densa (MD) (61). Succinate increases BP by binding to Gpr91 on the MD cells, which leads to renin release from the adjacent juxtaglomerular apparatus, thus driving the increase in BP (13) (Figure 2). Renin is an enzyme that cleaves angiotensinogen to form angiotensin 1, and this reaction constitutes the rate-limiting step in the RAS pathway. Because elevated succinate levels are associated with hypoxia and oxidative or mitochondrial stress, it may be that the kidney uses succinate as a “cue” to increase systemic BP (and, thus, its own perfusion) in the face of renal energy deprivation.

Intriguingly, this Gpr91–renin-angiotensin signaling pathway has been implicated in the pathophysiology of diabetic nephropathy. It has been appreciated for some time that diabetic nephropathy is a feature of both type 1 and type 2 diabetes, and that pharmacologic blockade of the RAS can slow the progression of diabetic nephropathy in patients with type 1 and type 2 diabetes (62). However, the unifying mechanism underlying this synergy has not

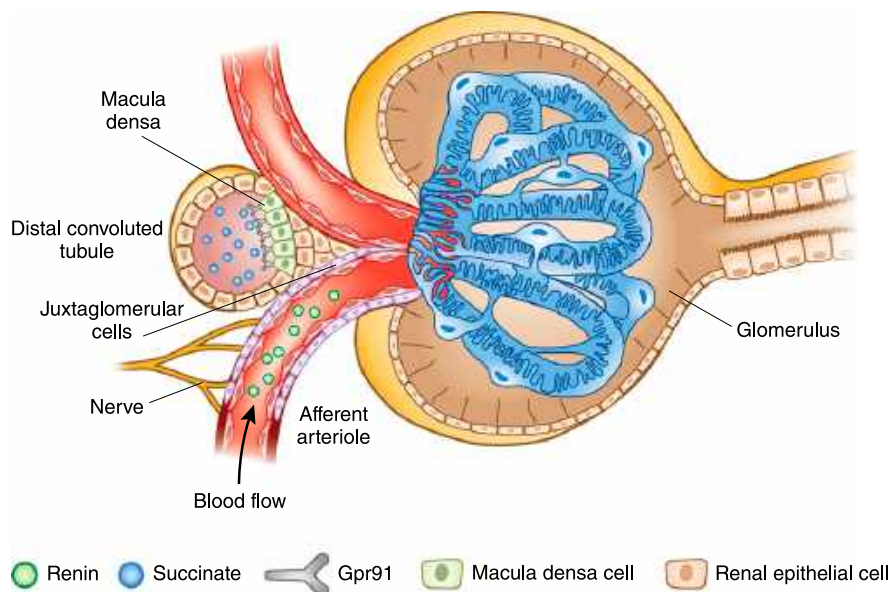


Figure 2. | Macula densa cell Gpr91 mediates renin release from juxtaglomerular cells in response to succinate binding. Gpr91, G protein–coupled receptor 91.

been well understood. The connection appears to be high plasma glucose levels, which are associated with elevated succinate levels in both kidney tissue and in urine (63). There is likely also increased localized succinate production (through the TCA cycle) in the kidney under conditions of hyperglycemia. These elevated succinate levels activate Gpr91, increasing renin release. It should be noted that although a minor component of the renin release response may be induced by the osmolar effects of high glucose levels, which is independent of Gpr91 (63), the majority of this response is Gpr91-mediated and succinate-dependent. Therefore, inappropriate activation of the RAS in diabetes is likely tied to elevated plasma glucose, and as a consequence, elevated succinate levels, which activate Gpr91 (and, therefore, renin release). These observations provide an interesting rationale for the well understood benefits of RAS blockade and plasma glucose control in the setting of diabetes.

Adenylate Cyclase 3

Adenylate cyclase 3 (AC3) is best known as a key component of the canonical signaling pathway underlying olfaction (64), but is also expressed in a variety of nonolfactory tissues (9,65), including the kidney (66). In the nose, when an odorant binds to its OR, OR activation leads to downstream activation of the olfactory G protein (G_{olf}), which, in turn, activates AC3, leading to the opening of nucleotide-gated ion channels, and, ultimately, to an action potential (6,64,67). AC3 is indispensable for the signaling pathway underlying olfaction, as evidenced by the fact that AC3 knockout mice are anosmic (64).

Within the kidney, AC3 colocalizes with G_{olf} in the distal convoluted tubule and the MD. The MD is a highly specialized, tightly packed cell type that occurs where each nephron makes physical contact with the vascular pole of its own parent glomerulus. At the junction between the thick ascending limb and the distal convoluted tubule, the renal epithelial cells lining the tubule are referred to as MD cells. These cells have a specialized sensory function that permits them to use chemical cues to deduce the flow rate of the tubular fluid in the distal nephron. These cells measure the chloride concentration of the tubular fluid, which is higher when the flow rate through the thick ascending limb is high. The MD cells can then signal the parent glomerulus to increase or decrease the flow rate, serving as a continuously operating single nephron GFR calibrator, in a process known as tubuloglomerular feedback. In addition, when flow rates are low, MD cells can also signal the juxtaglomerular cells associated with the afferent arteriole that feeds their parent glomerulus to increase renin secretion, which then raises systemic BP. Consistent with the role of the MD cells in tubuloglomerular feedback (and, therefore, GFR regulation) and renin secretion, knockout mice that do not express AC3 have decreased GFR and decreased plasma renin (66) levels, consistent with a defect in MD function. It remains to be determined which receptor and ligand signal through AC3 in the cells of the MD. It is clear, however, that components of the molecular machinery of olfaction play a key role in the kidney to regulate both GFR and renin secretion.

Short Chain Fatty Acids

The succinate pathway nicely exemplifies the kidney's use of sensory receptors to monitor intermediates and by-products of the body's own metabolism. Recent studies support the surprising conclusion that the kidney also appears to pay attention to metabolic by-products produced by the gut microbiota. Indeed, the number of cells contained within the human microbiota outnumbers the number of human cells in our bodies by more than a factor of 10 (68–71). The colon is the home to >70% of these microbes (69,71). A major class of metabolites produced by the gut microbiota in the colon are SCFAs (primarily acetate, propionate, and butyrate), which are produced at such a high rate that SCFAs are found in the colon at concentrations of approximately 100 mM (72). After absorption into the bloodstream, SCFAs are found in the plasma at concentrations ranging between 0.1 and 10 mM (73–75). SCFAs produced by gut microbes have recently emerged as signaling molecules in the physiology of the host, where they play roles in modulating physiologic processes, such as metabolism, immune responses, and susceptibility to HIV infection (73,74,76–79).

Gpr41 and Gpr43 are well characterized SCFA receptors (73,74,77) that have been shown to play important roles in regulating the physiology of the host organism in response to gut microbiota metabolite production. For example, in response to changes in plasma SCFA concentrations, Gpr41 regulates host adiposity (77), and Gpr43 modulates host inflammatory responses (73,74). It was recently found that both Gpr41 and Gpr43 are expressed in the kidney and the vasculature, along with a novel SCFA receptor, OR 78 (Olf78).

The identification and localization of Olf78 was particularly intriguing, because it was found in the kidney specifically in the afferent arteriole (a key component of the juxtaglomerular apparatus [JGA], which regulates renin secretion), as well as in resistance vasculature beds (80). The identification of Olf78 as a SCFA receptor (80), together with the distribution of Olf78 (80), led to the novel hypothesis that SCFA receptors may be responding to gut microbiota-derived SCFAs in order to regulate BP. Indeed, it was found that Olf78 is responsible for inducing renin release from the JGA upon increases in SCFAs (80) (Figure 3). This effect is detected in isolated JGA cells in culture, and Olf78 knockout mice exhibit reduced levels of plasma renin and resting BPs. In addition, a separate acute vascular effect of SCFAs was identified: SCFAs delivered intravenously cause a rapid and dose-dependent hypotensive response. This acute vascular effect was found to be mediated primarily by Gpr41, and to be opposed by a milder counter-regulatory hypertensive response to SCFAs driven by Olf78. In summary, SCFAs have an acute effect to lower BP *via* Gpr41, which is opposed by Olf78 in two ways: first, by an acute effect of Olf78 to support vascular tone; and second, by a more “chronic” effect of SCFAs to increase renin secretion *via* Olf78.

The functional significance of this pathway may be to enhance the capacity of the colon to maximally absorb remaining nutrients after a meal. Although most nutrient absorption occurs in the small intestine, a significant quantity of nutrients are absorbed from the large intestine in animals (72,81–89) and in humans (90–98). After a meal, one would expect SCFA

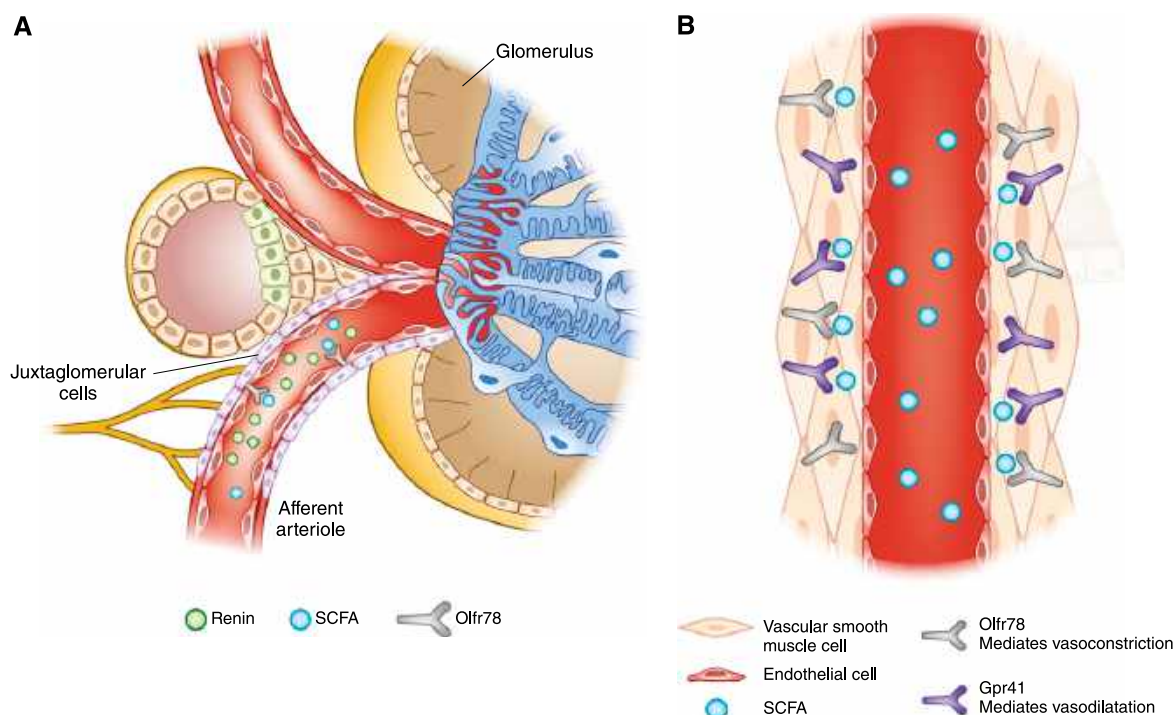


Figure 3. | SCFA Receptors and blood pressure. (A) Olfr78 mediates renin release from juxtaglomerular apparatus cells in response to binding of SCFAs. (B) Two different SCFA receptors (Olfr78 and Gpr41) are both expressed in blood vessels. Olfr78 mediates vasoconstriction in response to SCFAs, whereas Gpr41 mediates vasodilation. It should be noted that, whereas Olfr78 has been localized to vascular smooth muscle cells, Gpr41 has not been localized to a specific cell type within blood vessels (it was identified in blood vessels by RT-PCR). Therefore, although it is depicted here in vascular smooth muscle, the precise cell type in which it is localized has not been determined. Gpr41, G protein–coupled receptor 41; Olfr78, olfactory receptor 78; SCFA, short chain fatty acid.

concentrations in the vessels serving the large intestine to peak, driving localized vasodilation that would in turn facilitate nutrient absorption.

It should be noted that Gpr41 and Olfr78 have very different EC_{50} s (half maximal effective concentrations) for SCFAs [propionate: Gpr41, EC_{50} 100–300 μ M (73,99) in a guanosine 5'-O-(3-[^{35} S]thio)triphosphate binding assay; Olfr78, EC_{50} 0.9 mM (80) in a cAMP-based reporter assay]. Therefore, under basal conditions in which plasma SCFAs are 0.1–10 mM (73–75), one would expect Gpr41, but not Olfr78, to be tonically active. After a meal, however, as plasma SCFA concentrations rise, Gpr41 would become further active and, thus, would further lower BP until such a point that SCFA concentrations are sufficient to activate Olfr78 as well. Activation of Olfr78 would act as a “brake” on the hypotensive pathway, supporting an increase in BP and, therefore, preventing dangerous hypotension when SCFA concentrations are high.

These observations imply that BP is regulated not only by the genetics of an individual, but potentially by the genetics of that individual's microbiota as well. The potential clinical implications are numerous and prompt a variety of interesting questions. For example, do alterations in the diet of the host alter gut flora metabolite production and therefore BP? Can purposeful alteration of the gut microbiota composition alter BP? Similarly, are gut microbiota altered secondary to other clinical treatments (*e.g.*, antibiotics), and might this influence BP regulation? Future studies will be required to

better understand the complex and unexpected ways in which gut microbiota affect host physiology in general, and the renal regulation of BP in particular.

Bicarbonate

The proximal and distal segments of the renal tubule utilize distinct sensory mechanisms to detect the concentration of the bicarbonate ion and to tune ion transport processes accordingly. Bicarbonate transport in the proximal tubule is governed by several physiologic regulatory mediators, including angiotensin II, which acts through its GPCR to increase the activity of the apical sodium proton exchanger NHE3 and, thus, to increase bicarbonate reabsorption (100). Bicarbonate itself also appears to serve as a regulatory signal that modulates the activity of proximal tubule transporters. Although the molecular nature of the receptor that detects and responds to bicarbonate has yet to be determined, the activity of a tyrosine kinase appears to be involved in this process (101,102).

In more distal segments of the renal tubule, an ion transport protein appears to serve as a key component of a bicarbonate sensory mechanism that regulates sodium transport. Pendrin is a Na^+ -independent Cl^-/HCO_3^- exchanger expressed in the cochlea of the ear, the thyroid gland, and the kidney. In the kidney, pendrin plays a role in sensing and maintaining both volume status and acid-base balance. The gene encoding pendrin (solute carrier family 26, member 4 [SLC26A4]) is mutated in the

autosomal recessive disorder known as Pendred syndrome. The pathophysiology of Pendred syndrome reflects the tissue localization of the pendrin protein, as patients typically have hearing loss, goiter, and hypothyroidism. Pendrin mediates iodide flux in the thyroid (103), and it plays a key role in generating the endocochlear potential in the inner ear (104). Although patients with Pendred syndrome do not typically present with a clinically significant renal phenotype, recent studies have shown that pendrin does indeed play important roles in renal physiology. In the kidney, pendrin is expressed in the collecting duct, where it localizes apically in intercalated cells (105)—in particular, in type B and non-A non-B intercalated cells (105,106). The transport activity of pendrin in these cells appears to serve two distinct purposes. In its most straightforward role, pendrin is a coupled cotransport protein that mediates both Cl^- reabsorption and HCO_3^- secretion (107) and, thus, contributes to the collecting duct's compendium of ion transport machinery. Pendrin activity is upregulated by angiotensin II (108) and aldosterone (109). By mediating Cl^- absorption in the collecting duct, pendrin plays a role in volume homeostasis and, therefore, in BP control (109–111). As a direct consequence of chloride/bicarbonate exchange activity, however, pendrin also plays a central role in a recently defined signaling pathway. By modulating the bicarbonate concentration in the lumen of the collecting duct, pendrin sends a message to the neighboring principal cells that, in turn, regulates the functional expression of the ENaC sodium channels.

Evidence in support of this conclusion derives from observations made on mice that are deficient for pendrin expression. Although pendrin knockout mice are normal at baseline, they do not gain weight and do not become hypertensive upon treatment with an aldosterone analog (unlike their wild-type counterparts) (109). If, however, the bicarbonate concentration of the collecting duct tubule fluid in the pendrin knockout mice is raised artificially through pharmacologic manipulations, then these animals respond normally to aldosterone by increasing their ENaC-mediated sodium absorption. It appears that the bicarbonate ions that pendrin secretes into the collecting duct lumen act through an as-yet-mysterious signaling pathway to stimulate the upregulation of ENaC expression and activity in principal cells (107).

In addition, pendrin also acts as the “downstream” partner in a paracrine signaling scheme. A recent study demonstrated that pendrin activity is altered by changes in proximal tubule transport (112). It was reported that changes in acid-base status alter proximal tubule transport of α -ketoglutarate (α KG), and lead to changes in the concentration of α KG in the distal tubular fluid. In the connecting tubule and collecting ducts, α KG is sensed in intercalated cells by a receptor (oxoglutarate receptor 1), which then stimulates pendrin. Therefore, although pendrin itself is not found in the proximal tubule, it is responsive to acid-base effects on proximal tubule transport.

Thus, pendrin appears to serve in a variety of physiologic functions. By mediating Cl^- absorption, pendrin collaborates with ENaC to participate in transepithelial NaCl uptake, thus playing a direct role in regulating volume status. By mediating HCO_3^- secretion, pendrin participates directly in the regulation of acid-base balance. It also determines the

luminal concentration of bicarbonate, which acts as the critical determinant in a chemosensory pathway that helps to control the capacity of the principal cells to increase their sodium absorptive capacity in response to aldosterone. Finally, pendrin activity is also regulated by the acid-base status of the proximal tubule. Although many of the receptors and signaling pathways involved in this bicarbonate chemosensory process have yet to be elucidated, these schemes beautifully illustrate a fascinating cross-talk between transport and sensory processes in the kidney.

The kidney must respond to a wide variety of physiologic cues in its efforts to maintain homeostasis. It has only recently been appreciated that both mechanosensors and chemosensors play important roles in controlling a variety of renal functions in response to a number of novel stimuli. Future studies will no doubt provide us with a clearer understanding of the physiologic and pathophysiologic implications of these pathways in the governance of renal function.

Disclosures

M.J.C is a consultant for Allertein Pharmaceuticals and Portage Pharmaceuticals, serves on the Telethon Foundation Scientific Advisory Board, and is an editorial consultant for Elsevier Publishing.

References

- Huang AL, Chen X, Hoon MA, Chandrashekar J, Guo W, Tränkner D, Ryba NJ, Zuker CS: The cells and logic for mammalian sour taste detection. *Nature* 442: 934–938, 2006
- Deshpande DA, Wang WC, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, Sham JS, Liggett SB: Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med* 16: 1299–1304, 2010
- Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ: Motile cilia of human airway epithelia are chemosensory. *Science* 325: 1131–1134, 2009
- Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, Kim HH, Xu X, Chan SL, Juhaszova M, Bernier M, Mosinger B, Margolskee RF, Egan JM: Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci U S A* 104: 15069–15074, 2007
- Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, Shirazi-Beechey SP: T1R3 and gustducin in gut sense sugars to regulate expression of Na⁺-glucose cotransporter 1. *Proc Natl Acad Sci U S A* 104: 15075–15080, 2007
- Buck L, Axel R: A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* 65: 175–187, 1991
- Malnic B, Godfrey PA, Buck LB: The human olfactory receptor gene family. *Proc Natl Acad Sci U S A* 101: 2584–2589, 2004
- Godfrey PA, Malnic B, Buck LB: The mouse olfactory receptor gene family. *Proc Natl Acad Sci U S A* 101: 2156–2161, 2004
- Griffin CA, Kafadar KA, Pavlath GK: MOR23 promotes muscle regeneration and regulates cell adhesion and migration. *Dev Cell* 17: 649–661, 2009
- Magistroni R, Furci L, Albertazzi A: [Autosomal dominant polycystic kidney disease: From genes to cilium]. *G Ital Nefrol* 25: 183–191, 2008
- He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, Chen JL, Tian H, Ling L: Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 429: 188–193, 2004
- Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, Kobayashi M, Hirasawa A, Tsujimoto G: Short-chain fatty acids

- and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A* 108: 8030–8035, 2011
13. Vargas SL, Toma I, Kang JJ, Meer EJ, Peti-Peterdi J: Activation of the succinate receptor GPR91 in macula densa cells causes renin release. *J Am Soc Nephrol* 20: 1002–1011, 2009
 14. Weinbaum S, Duan Y, Satlin LM, Wang T, Weinstein AM: Mechanotransduction in the renal tubule. *Am J Physiol Renal Physiol* 299: F1220–F1236, 2010
 15. Aigouy B, Farhadifar R, Staple DB, Sagner A, Röper JC, Jülicher F, Eaton S: Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell* 142: 773–786, 2010
 16. Fischer E, Legue E, Doyen A, Nato F, Nicolas JF, Torres V, Yaniv M, Pontoglio M: Defective planar cell polarity in polycystic kidney disease. *Nat Genet* 38: 21–23, 2006
 17. Kramer-Zucker AG, Olale F, Haycraft CJ, Yoder BK, Schier AF, Drummond IA: Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. *Development* 132: 1907–1921, 2005
 18. Berrout J, Jin M, Mamenko M, Zaika O, Pochynyuk O, O'Neil RG: Function of transient receptor potential cation channel subfamily V member 4 (TRPV4) as a mechanical transducer in flow-sensitive segments of renal collecting duct system. *J Biol Chem* 287: 8782–8791, 2012
 19. O'Neil RG, Heller S: The mechanosensitive nature of TRPV channels. *Pflügers Arch* 451: 193–203, 2005
 20. Holtzclaw JD, Cornelius RJ, Hatcher LI, Sansom SC: Coupled ATP and potassium efflux from intercalated cells. *Am J Physiol Renal Physiol* 300: F1319–F1326, 2011
 21. Holtzclaw JD, Liu L, Grimm PR, Sansom SC: Shear stress-induced volume decrease in C11-MDCK cells by BK- α /beta4. *Am J Physiol Renal Physiol* 299: F507–F516, 2010
 22. Liu W, Xu S, Woda C, Kim P, Weinbaum S, Satlin LM: Effect of flow and stretch on the $[Ca^{2+}]_i$ response of principal and intercalated cells in cortical collecting duct. *Am J Physiol Renal Physiol* 285: F998–F1012, 2003
 23. Satlin LM, Sheng S, Woda CB, Kleyman TR: Epithelial Na(+) channels are regulated by flow. *Am J Physiol Renal Physiol* 280: F1010–F1018, 2001
 24. Woda CB, Bragin A, Kleyman TR, Satlin LM: Flow-dependent K⁺ secretion in the cortical collecting duct is mediated by a maxi-K channel. *Am J Physiol Renal Physiol* 280: F786–F793, 2001
 25. Pluznick JL, Wei P, Grimm PR, Sansom SC: BK- β 1 subunit: Immunolocalization in the mammalian connecting tubule and its role in the kaliuretic response to volume expansion. *Am J Physiol Renal Physiol* 288: F846–F854, 2005
 26. Ortiz PA, Hong NJ, Garvin JL: Luminal flow induces eNOS activation and translocation in the rat thick ascending limb. II. Role of PI3-kinase and Hsp90. *Am J Physiol Renal Physiol* 287: F281–F288, 2004
 27. Ortiz PA, Hong NJ, Garvin JL: Luminal flow induces eNOS activation and translocation in the rat thick ascending limb. *Am J Physiol Renal Physiol* 287: F274–F280, 2004
 28. Chauvet V, Tian X, Husson H, Grimm DH, Wang T, Hiesberger T, Igarashi P, Bennett AM, Ibraghimov-Beskrovnaya O, Somlo S, Caplan MJ: Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. *J Clin Invest* 114: 1433–1443, 2004
 29. Rice WL, Van Hoek AN, Păunescu TG, Huynh C, Goetze B, Singh B, Scipioni L, Stern LA, Brown D: High resolution helium ion scanning microscopy of the rat kidney. *PLoS ONE* 8: e57051, 2013
 30. Wang S, Dong Z: Primary cilia and kidney injury: Current research status and future perspectives. *Am J Physiol Renal Physiol* 305: F1085–F1098, 2013
 31. Satir P, Christensen ST: Overview of structure and function of mammalian cilia. *Annu Rev Physiol* 69: 377–400, 2007
 32. Pedersen LB, Rosenbaum JL: Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling. *Curr Top Dev Biol* 85: 23–61, 2008
 33. Qin H, Diener DR, Geimer S, Cole DG, Rosenbaum JL: Intraflagellar transport (IFT) cargo: IFT transports flagellar precursors to the tip and turnover products to the cell body. *J Cell Biol* 164: 255–266, 2004
 34. Szymanska K, Johnson CA: The transition zone: An essential functional compartment of cilia. *Cilia* 1: 10, 2012
 35. Hu Q, Nelson WJ: Ciliary diffusion barrier: The gatekeeper for the primary cilium compartment. *Cytoskeleton (Hoboken)* 68: 313–324, 2011
 36. Pazour GJ, Rosenbaum JL: Intraflagellar transport and cilia-dependent diseases. *Trends Cell Biol* 12: 551–555, 2002
 37. Gascue C, Katsanis N, Badano JL: Cystic diseases of the kidney: Ciliary dysfunction and cystogenic mechanisms. *Pediatr Nephrol* 26: 1181–1195, 2011
 38. Waters AM, Beales PL: Ciliopathies: An expanding disease spectrum. *Pediatr Nephrol* 26: 1039–1056, 2011
 39. Yoder BK, Hou X, Guay-Woodford LM: The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol* 13: 2508–2516, 2002
 40. Pazour GJ, San Agustin JT, Folliot JA, Rosenbaum JL, Witman GB: Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr Biol* 12: R378–R380, 2002
 41. Chapin HC, Caplan MJ: The cell biology of polycystic kidney disease. *J Cell Biol* 191: 701–710, 2010
 42. Praetorius HA, Spring KR: The renal cell primary cilium functions as a flow sensor. *Curr Opin Nephrol Hypertens* 12: 517–520, 2003
 43. Köttgen M, Buchholz B, Garcia-Gonzalez MA, Kotsis F, Fu X, Doerken M, Boehlke C, Steffl D, Tauber R, Wegierski T, Nitschke R, Suzuki M, Kramer-Zucker A, Germino GG, Watnick T, Prenen J, Nilius B, Kuehn EW, Walz G: TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol* 182: 437–447, 2008
 44. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33: 129–137, 2003
 45. DeCaen PG, Delling M, Vien TN, Clapham DE: Direct recording and molecular identification of the calcium channel of primary cilia. *Nature* 504: 315–318, 2013
 46. Delling M, DeCaen PG, Doerner JF, Febray S, Clapham DE: Primary cilia are specialized calcium signalling organelles. *Nature* 504: 311–314, 2013
 47. Parnell SC, Magenheimer BS, Maser RL, Rankin CA, Smine A, Okamoto T, Calvet JP: The polycystic kidney disease-1 protein, polycystin-1, binds and activates heterotrimeric G-proteins in vitro. *Biochem Biophys Res Commun* 251: 625–631, 1998
 48. LopezJimenez ND, Cavenagh MM, Sainz E, Cruz-Ithier MA, Battey JF, Sullivan SL: Two members of the TRPP family of ion channels, Pkd1l3 and Pkd2l1, are co-expressed in a subset of taste receptor cells. *J Neurochem* 98: 68–77, 2006
 49. Ishii S, Misaka T, Kishi M, Kaga T, Ishimaru Y, Abe K: Acetic acid activates PKD1L3-PKD2L1 channel—a candidate sour taste receptor. *Biochem Biophys Res Commun* 385: 346–350, 2009
 50. Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S: Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nat Genet* 45: 1004–1012, 2013
 51. Praetorius HA, Leipziger J: Primary cilium-dependent sensing of urinary flow and paracrine purinergic signaling. *Semin Cell Dev Biol* 24: 3–10, 2013
 52. Bjælde RG, Arnadóttir SS, Overgaard MT, Leipziger J, Praetorius HA: Renal epithelial cells can release ATP by vesicular fusion. *Front Physiol* 4: 238, 2013
 53. Booth JW, Tam FW, Unwin RJ: P2 purinoceptors: Renal pathophysiology and therapeutic potential. *Clin Nephrol* 78: 154–163, 2012
 54. Unwin RJ, Bailey MA, Burnstock G: Purinergic signaling along the renal tubule: The current state of play. *News Physiol Sci* 18: 237–241, 2003
 55. Wildman SS, Marks J, Turner CM, Yew-Booth L, Peppiatt-Wildman CM, King BF, Shirley DG, Wang W, Unwin RJ: Sodium-dependent regulation of renal amiloride-sensitive currents by apical P2 receptors. *J Am Soc Nephrol* 19: 731–742, 2008
 56. Liu W, Wei Y, Sun P, Wang WH, Kleyman TR, Satlin LM: Mechanoregulation of BK channel activity in the mammalian

- cortical collecting duct: Role of protein kinases A and C. *Am J Physiol Renal Physiol* 297: F904–F915, 2009
57. Duan Y, Weinstein AM, Weinbaum S, Wang T: Shear stress-induced changes of membrane transporter localization and expression in mouse proximal tubule cells. *Proc Natl Acad Sci U S A* 107: 21860–21865, 2010
 58. Peti-Peterdi J: High glucose and renin release: The role of succinate and GPR91. *Kidney Int* 78: 1214–1217, 2010
 59. Goldberg ND, Passonneau JV, Lowry OH: Effects of changes in brain metabolism on the levels of citric acid cycle intermediates. *J Biol Chem* 241: 3997–4003, 1966
 60. Hebert SC: Physiology: Orphan detectors of metabolism. *Nature* 429: 143–145, 2004
 61. Robben JH, Fenton RA, Vargas SL, Schweer H, Peti-Peterdi J, Deen PM, Milligan G: Localization of the succinate receptor in the distal nephron and its signaling in polarized MDCK cells. *Kidney Int* 76: 1258–1267, 2009
 62. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P: Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345: 870–878, 2001
 63. Toma I, Kang JJ, Sipos A, Vargas S, Bansal E, Hanner F, Meer E, Peti-Peterdi J: Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *J Clin Invest* 118: 2526–2534, 2008
 64. Wong ST, Trinh K, Hacker B, Chan GC, Lowe G, Gaggar A, Xia Z, Gold GH, Storm DR: Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* 27: 487–497, 2000
 65. Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK, Hatt H: Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* 299: 2054–2058, 2003
 66. Pluznick JL, Zou DJ, Zhang X, Yan Q, Rodriguez-Gil DJ, Eisner C, Wells E, Greer CA, Wang T, Firestein S, Schnermann J, Caplan MJ: Functional expression of the olfactory signaling system in the kidney. *Proc Natl Acad Sci U S A* 106: 2059–2064, 2009
 67. Jones DT, Reed RR: Golf: An olfactory neuron specific-G protein involved in odorant signal transduction. *Science* 244: 790–795, 1989
 68. Sekirov I, Russell SL, Antunes LC, Finlay BB: Gut microbiota in health and disease. *Physiol Rev* 90: 859–904, 2010
 69. Ley RE, Peterson DA, Gordon JL: Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124: 837–848, 2006
 70. Savage DC: Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 31: 107–133, 1977
 71. Whitman WB, Coleman DC, Wiebe WJ: Prokaryotes: The unseen majority. *Proc Natl Acad Sci U S A* 95: 6578–6583, 1998
 72. Bugaut M: Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comp Biochem Physiol B* 86: 439–472, 1987
 73. Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M: Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem* 278: 25481–25489, 2003
 74. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR: Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461: 1282–1286, 2009
 75. Samuel BS, Gordon JL: A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. *Proc Natl Acad Sci U S A* 103: 10011–10016, 2006
 76. Hill MJ, Drasar BS: The normal colonic bacterial flora. *Gut* 16: 318–323, 1975
 77. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JL: Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci U S A* 105: 16767–16772, 2008
 78. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JL: A core gut microbiome in obese and lean twins. *Nature* 457: 480–484, 2009
 79. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JL, Krakoff J: Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr* 94: 58–65, 2011
 80. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, Firestein SJ, Yanagisawa M, Gordon JL, Eichmann A, Peti-Peterdi J, Caplan MJ: Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci U S A* 110: 4410–4415, 2013
 81. Armstrong DG, Beever DE: Post-abomasal digestion of carbohydrate in the adult ruminant. *Proc Nutr Soc* 28: 121–131, 1969
 82. Demigne C, Remesy C: Influence of unrefined potato starch on cecal fermentations and volatile fatty acid absorption in rats. *J Nutr* 112: 2227–2234, 1982
 83. Demigné C, Rémesy C, Rayssiguier Y: Effect of fermentable carbohydrates on volatile fatty acids, ammonia and mineral absorption in the rat caecum. *Reprod Nutr Dev* 20: 1351–1359, 1980
 84. Hintz HF, Hogue DE, Walker EF Jr, Lowe JE, Schryver HF: Apparent digestion in various segments of the digestive tract of ponies fed diets with varying roughage-grain ratios. *J Anim Sci* 32: 245–248, 1971
 85. Keys JE Jr, DeBarthe JV: Site and extent of carbohydrate, dry matter, energy and protein digestion and the rate of passage of grain diets in swine. *J Anim Sci* 39: 57–62, 1974
 86. Orskov ER, Fraser C, Kay RN: Dietary factors influencing the digestion of starch in the rumen and small and large intestine of early weaned lambs. *Br J Nutr* 23: 217–226, 1969
 87. Orskov ER, Fraser C, McDonald I: Digestion of concentrates in sheep. 3. Effects of rumen fermentation of barley and maize diets on protein digestion. *Br J Nutr* 26: 477–486, 1971
 88. Orskov ER, Fraser C, Mason VC, Mann SO: Influence of starch digestion in the large intestine of sheep on caecal fermentation, caecal microflora and faecal nitrogen excretion. *Br J Nutr* 24: 671–682, 1970
 89. Topping DL, Illman RJ, Taylor MN, McIntosh GH: Effects of wheat bran and porridge oats on hepatic portal venous volatile fatty acids in the pig. *Ann Nutr Metab* 29: 325–331, 1985
 90. Anderson IH, Levine AS, Levitt MD: Incomplete absorption of the carbohydrate in all-purpose wheat flour. *N Engl J Med* 304: 891–892, 1981
 91. Bond JH, Levitt MD: Quantitative measurement of lactose absorption. *Gastroenterology* 70: 1058–1062, 1976
 92. Englyst HN, Cummings JH: Digestion of the polysaccharides of some cereal foods in the human small intestine. *Am J Clin Nutr* 42: 778–787, 1985
 93. McNeil NI, Bingham S, Cole TJ, Grant AM, Cummings JH: Diet and health of people with an ileostomy. 2. Ileostomy function and nutritional state. *Br J Nutr* 47: 407–415, 1982
 94. Perman JA, Modler S: Role of the intestinal microflora in disposition of nutrients in the gastrointestinal tract. *J Pediatr Gastroenterol Nutr* 2[Suppl 1]: S193–S196, 1983
 95. Pomare EW, Branch WJ, Cummings JH: Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. *J Clin Invest* 75: 1448–1454, 1985
 96. Sandberg AS, Andersson H, Kivistö B, Sandström B: Extrusion cooking of a high-fibre cereal product. 1. Effects on digestibility and absorption of protein, fat, starch, dietary fibre and phytate in the small intestine. *Br J Nutr* 55: 245–254, 1986
 97. Saunders DR, Wiggins HS: Conservation of mannitol, lactulose, and raffinose by the human colon. *Am J Physiol* 241: G397–G402, 1981
 98. Wiggins HS: Nutritional value of sugars and related compounds undigested in the small gut. *Proc Nutr Soc* 43: 69–75, 1984
 99. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ: The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* 278: 11312–11319, 2003
 100. Capasso G, Unwin R, Rizzo M, Pica A, Giebisch G: Bicarbonate transport along the loop of Henle: Molecular mechanisms and regulation. *J Nephrol* 15[Suppl 5]: S88–S96, 2002

101. Zhao J, Zhou Y, Boron WF: Effect of isolated removal of either basolateral HCO₃⁻ or basolateral CO₂ on HCO₃⁻ reabsorption by rabbit S2 proximal tubule. *Am J Physiol Renal Physiol* 285: F359–F369, 2003
102. Zhou Y, Bouyer P, Boron WF: Role of a tyrosine kinase in the CO₂-induced stimulation of HCO₃⁻ reabsorption by rabbit S2 proximal tubules. *Am J Physiol Renal Physiol* 291: F358–F367, 2006
103. Scott DA, Wang R, Kreman TM, Sheffield VC, Karniski LP: The Pendred syndrome gene encodes a chloride-iodide transport protein. *Nat Genet* 21: 440–443, 1999
104. Everett LA, Belyantseva IA, Noben-Trauth K, Cantos R, Chen A, Thakkar SI, Hoogstraten-Miller SL, Kachar B, Wu DK, Green ED: Targeted disruption of mouse Pds provides insight about the inner-ear defects encountered in Pendred syndrome. *Hum Mol Genet* 10: 153–161, 2001
105. Royaux IE, Wall SM, Karniski LP, Everett LA, Suzuki K, Knepper MA, Green ED: Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc Natl Acad Sci U S A* 98: 4221–4226, 2001
106. Wall SM, Hassell KA, Royaux IE, Green ED, Chang JY, Shipley GL, Verlander JW: Localization of pendrin in mouse kidney. *Am J Physiol Renal Physiol* 284: F229–F241, 2003
107. Pech V, Pham TD, Hong S, Weinstein AM, Spencer KB, Duke BJ, Walp E, Kim YH, Sutliff RL, Bao HF, Eaton DC, Wall SM: Pendrin modulates ENaC function by changing luminal HCO₃⁻. *J Am Soc Nephrol* 21: 1928–1941, 2010
108. Verlander JW, Hong S, Pech V, Bailey JL, Agazatian D, Matthews SW, Coffman TM, Le T, Inagami T, Whitehill FM, Weiner ID, Farley DB, Kim YH, Wall SM: Angiotensin II acts through the angiotensin 1a receptor to upregulate pendrin. *Am J Physiol Renal Physiol* 301: F1314–F1325, 2011
109. Verlander JW, Hassell KA, Royaux IE, Glapion DM, Wang ME, Everett LA, Green ED, Wall SM: Deoxycorticosterone upregulates PDS (Slc26a4) in mouse kidney: Role of pendrin in mineralocorticoid-induced hypertension. *Hypertension* 42: 356–362, 2003
110. Wall SM, Pech V: Pendrin and sodium channels: Relevance to hypertension. *J Nephrol* 23[Suppl 16]: S118–S123, 2010
111. Wall SM, Weinstein AM: Cortical distal nephron Cl⁻ transport in volume homeostasis and blood pressure regulation. *Am J Physiol Renal Physiol* 305: F427–F438, 2013
112. Tokonami N, Morla L, Centeno G, Mordasini D, Ramakrishnan SK, Nikolaeva S, Wagner CA, Bonny O, Houillier P, Doucet A, Firsov D: α -Ketoglutarate regulates acid-base balance through an intrarenal paracrine mechanism. *J Clin Invest* 123: 3166–3171, 2013

Published online ahead of print. Publication date available at www.cjasn.org.



Physiology of the Renal Interstitium

Michael Zeisberg* and Raghu Kalluri†

Abstract

Long overlooked as the virtual compartment and then strictly characterized through descriptive morphologic analysis, the renal interstitium has finally been associated with function. With identification of interstitial renin- and erythropoietin-producing cells, the most prominent endocrine functions of the kidney have now been attributed to the renal interstitium. This article reviews the functional role of renal interstitium.

Clin J Am Soc Nephrol 10: 1831–1840, 2015. doi: 10.2215/CJN.00640114

Introduction

The physiologic role of the interstitium of the kidney has received comparatively little interest to date. This may partially be attributed to the fact that it was long considered the exclusive domain of descriptive ultrastructural research, which implied that the interstitium was mostly a passive tissue that structurally supported the tubular epithelium (1,2). The renal interstitium received increasing interest in the context of kidney abnormalities, when the role of interstitial fibrosis in progression of CKD became obvious (3–5). Only since transgenic animal studies revealed the physiologic endocrine function of interstitial cells as sources of erythropoietin (Epo) and renin has the physiologic role of the renal interstitium received its due attention. Here we review current knowledge of the form and function of the renal interstitium.

Definition and Ultrastructure of the Renal Interstitium

The renal interstitium is defined as the intertubular, extraglomerular, extravascular space of the kidney. It is bounded on all sides by tubular and vascular basement membranes and is filled with cells, extracellular matrix, and interstitial fluid (1). Its distribution varies within the kidney; it accounts for approximately 8% of the total parenchymal volume in the cortex and up to 40% in the inner medulla (6,7). The term “renal interstitium” is often inadequately used to refer to the peritubular interstitium (the space between tubules, glomeruli, and capillaries); the periarterial connective tissue and the extraglomerular mesangium are considered specialized interstitia (1). It is debated whether microvessels and capillaries, which are located within the peritubular space, are actually part of the renal interstitium or just run through it (1). Furthermore, lymphatics are considered interstitial constituents (1). The tubular interstitium in the cortex and medulla differ with regard to their cellular constituents, extracellular matrix composition, relative volume, and endocrine function, justifying the consideration of cortical and medullary interstitium as separate entities.

The intertubular interstitium harbors dendritic cells, macrophages, lymphocytes, lymphatic endothelial cells

(in the cortical intertubular interstitium), and various types of fibroblasts, the hallmark cell type of connective tissues (8). In addition, it is believed that the interstitium plays a role in fluid and electrolyte exchange and insulation (1). Here we focus on fibroblasts and specifically on fibroblasts with endocrine function and their biology in health and disease (other articles have extensively reviewed cellular constituents of the immune system within the renal interstitium [9,10]) (Figure 1).

Renal Fibroblasts

In general, fibroblasts are flattened cells with extended cell processes; in profile they display a fusiform or spindle-like shape and a flattened nucleus (11). Fibroblasts typically are embedded within the fibrillar matrix of connective tissues and are considered prototypical mesenchymal cells. Renal fibroblasts anastomose with each other, forming a continuous network in cortex and medulla (8). Fibroblasts can acquire an activated phenotype with a relatively large oval nucleus with one or two nucleoli, abundant rough endoplasmic reticulum, and several sets of Golgi apparatus; this reflects their capacity to synthesize substantial amounts of extracellular matrix constituents (12). Under physiologic conditions, however, adult fibroblasts are relatively inactive, the endoplasmic reticulum is reduced, and the nucleus is flattened and heterochromatic. On the basis of their appearance, it was assumed that the primary function of renal fibroblasts was to provide structural support to nephrons through deposition of extracellular matrix and through direct cell-cell interactions (1). In addition, fibroblasts play an important role in maintaining vascular integrity in close association with vessels (then typically referred to as vascular smooth muscle cells and pericytes) (13,14). Renal fibroblasts are best known for their role in progression of interstitial fibrosis in progressive CKD because they are principal producers of extracellular matrix (4). Finally, fibroblasts have been identified as sources of Epo and renin in the kidney (15,16). It is obvious that such diverse functions are not fulfilled by one cell type but that renal fibroblasts are instead a heterogeneous cell population with distinct functions.

*Department of Nephrology and Rheumatology, Göttingen University Medical Center, Georg August University, Göttingen, Germany; and †Department of Cancer Biology and the Metastasis Research Center, University of Texas MD Anderson Cancer Center, Houston, Texas

Correspondence:

Dr. Michael Zeisberg, Department of Nephrology and Rheumatology, Göttingen University Medical Center, Georg August University, Robert Koch Str. 40, 37075 Göttingen, Germany. Email: mzeisberg@med.uni-goettingen.de.
Dr. Raghu Kalluri, Department of Cancer Biology and the Metastasis Research Center, MD Anderson Cancer Center, 1901 East Road, Houston, TX 77054. Email: rkalluri@mdanderson.org

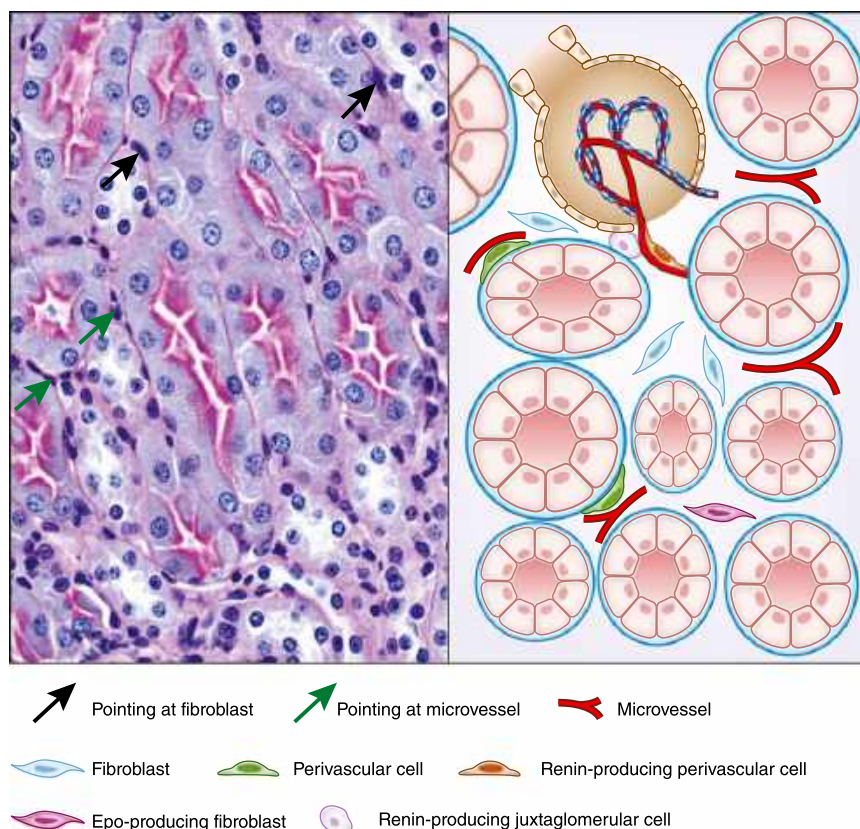


Figure 1. | Content of the virtual compartment. Left. The photomicrograph displays a cortical section of a paraffin-fixed periodic acid-Schiff-stained mouse kidney. The interstitium, located between tubules, is barely visible. Arrows (black) point to interstitial fibroblasts or to microvessels, respectively (green). Right. Glomerular and tubular cross-sections and contents of the interstitium are illustrated in the schematic. The interstitial compartment contains non-hormone-producing fibroblasts (blue), microvessels (red), perivascular cells (green), renin-producing perivascular cells (orange), juxtaglomerular cells (lilac), and erythropoietin (Epo)-producing fibroblasts (pink). Bottom. Representative drawings are explained.

Heterogeneity of Renal Fibroblasts

Our current knowledge of fibroblast heterogeneity evolved from morphologic descriptions to immunophenotyping, and only recently the use of transgenic mouse models provided insights into origins and functions of renal fibroblasts. Ultrastructural analysis first revealed that cortical fibroblasts differ from medullary fibroblasts, as they form a finer network through their radiating cytoplasmic processes with tubular cells, endothelial cells, and each other. Furthermore, medullary fibroblasts often have a stouter appearance and harbor cytoplasmatic lipid droplets (8). However, such ultrastructural analysis revealed little about distinct functions of fibroblast populations (1,2,8,17). Attempts to link ultrastructural appearance to function were followed by studies that aimed to define renal fibroblast populations through use of fibroblast markers. Such fibroblast markers included the intermediate filament-associated protein vimentin (which is not specific for fibroblasts because it is also present in endothelial cells and neurons) (18); desmin (which is present only in a subset of fibroblasts and in muscle cells) (19,20); collagen receptors, such as $\alpha1\beta1$ integrin (21) and discoidin domain receptor 2 (which are also present on cells of various other lineages, including endothelial cells) (22); the intracellular calcium-binding protein fibroblast-specific protein 1 (which is present on fibroblasts but also in invasive carcinoma cells) (23); the membrane-bound enzyme ecto-5'-nucleotidase

(CD73) (which labels cortical renal fibroblasts but also T-lymphocytes) (24,25); the PDGF receptor PDGF receptor- β (which labels fibroblasts but also macrophages) (26,27); and α -smooth muscle actin (which labels a subset of activated fibroblasts and vascular smooth muscle cells) (28). In addition, the synthesis of collagen types I, III, and V are considered a hallmark feature of fibroblasts, although many other cell types also robustly express these collagens (28). Clearly, all proposed markers had deficiencies, creating an as-yet unresolved dispute in the field regarding identity of fibroblasts and their respective function in kidney health and disease. The confusion is further fueled by assumed different origins of fibroblasts in diseased kidneys (for extensive information, see elsewhere [4,29]). For practical purposes, we here define fibroblasts as the nonvascular, nonepithelial, and noninflammatory cell constituents of the kidney (11). In the following paragraphs we discuss the role of fibroblasts as sources of Epo and renin in kidney health and disease.

Epo

Epo is an indispensable glycoprotein hormone that is produced by interstitial renal fibroblasts and controls hematopoiesis through promotion of survival, proliferation, and differentiation of erythroid progenitors (30). Epo is best known through its recombinant protein relatives, which

are widely used to treat anemia in dialysis patients (a \$12 billion market worldwide at its peak in 2006) and its misuse as a performance-enhancing substance in cycling and other sports (31). Insights into the physiologic mechanism that underlies production of endogenous Epo are of interest to understand the molecular basis of Epo deficiency in patients with CKD and potential therapeutic strategies to circumvent lack of Epo. Here we review the origins of endogenous Epo and molecular mechanisms that control Epo production in health and disease.

Identification of Renal Interstitial Fibroblasts as Primary Origin of Epo

In adults, about 90% of Epo is produced by renal interstitial fibroblasts, whereas 10% is contributed by extrarenal sources, primarily liver cells (32). Decreased oxygen tension is the principal stimulus for renal Epo expression, yet Epo production decreases in CKD, which is associated with chronic hypoxia (33,34). When Epo production is impaired in the injured kidney, extrarenal sources of Epo cannot compensate for decreased Epo levels (35). Nevertheless, despite decades of effort, Epo-producing kidney cells have not yet been cultivated, which is why most analyses of Epo production relied on liver-derived hepatoma cells (36). The value of using hepatoma cells to study mechanisms of Epo production is limited, however, because control of Epo expression underlies distinct mechanisms in kidney and liver. To better understand this unusual complexity of endogenous Epo expression, it is worth reviewing the sequence of discoveries that led to the knowledge of Epo expression.

A link between decreased oxygen availability and an adaptive increase in red blood cell count was first suspected by Miescher, who observed increased hemoglobin levels in patients who entered a high-altitude sanatorium in the Alps (37). Yet it was not until 1953 when Erslev demonstrated that plasma transfer from anemic rabbits caused increased hemoglobin levels (but not white blood cells) in recipient rabbits (38). Goldwasser succeeded in isolating Epo for the first time in 1977, but it took him 17 years and 2500 L of urine from patients with aplastic anemia to achieve this goal (39).

Because of the consistent association between CKD and anemia, the kidney was long suspected as a primary source of Epo. However, Epo expression levels are low, and immunolabeling and *in situ* hybridization analyses proved to be unreliable; it took extensive studies using transgenic mice to finally, unequivocally identify renal interstitial fibroblasts as primary producers of Epo (16,40–42). Furthermore, analysis of Epo expression *in vivo* is technically challenging because large regions of noncoding DNA flanking the Epo locus (which contains tissue-specific enhancers and repressors) must be included to adequately reflect endogenous Epo expression. Only recently, use of a 180-kb Epo transgene fused to a green fluorescent protein reporter tag unequivocally located hypoxia-induced Epo expression to fibroblasts in the deep cortex to the outer medulla region (41). The identity of these Epo-producing cells as fibroblasts was confirmed by immunophenotyping with antibodies to CD73.

Regulation of Epo Production

The kidney is the principal source of Epo. Because Epo deficiency is the most important component of the anemia that complicates CKD, cellular origins and molecular

mechanisms that control endogenous Epo production are of great clinical relevance (43). Physiologic plasma Epo levels in blood are relatively low at around 100 pg/ml, whereas under hypoxic stress associated with anemia, Epo levels can reach 100,000 pg/ml (44). A drop in tissue PO₂ is the principal stimulus for Epo expression, and PO₂ levels are consistently higher within the cortical interstitium because there is a constant ratio of blood flow rate and small O₂ consumption, relatively independent of cardiac output and renal blood flow (45,46). Hence, a drop in hemoglobin levels and ensuing tissue hypoxia are a prototypical stimulus for Epo expression. Because Epo is not stored and has a relatively short half-life, circulating Epo levels are closely linked to Epo expression in interstitial fibroblasts. The mechanisms that control transcriptional activity have received prominent attention in recent years (summarized in Figure 2) (44). The true value of understanding mechanisms that underlie control of Epo expression may be the possible therapeutic solutions to overcome the transcriptional silencing of Epo, a hallmark of CKD (47). While hypoxia is the principal stimulus for Epo expression under physiologic conditions, Epo transcription is silenced in chronically diseased kidneys, despite the hypoxic conditions that are a hallmark of renal fibrogenesis (34).

The molecular mechanisms that underlie transcriptional silencing of Epo in fibrotic fibroblasts is only incompletely understood, at least in part because of aforementioned difficulties in the study of Epo expression in cultured renal cells. Two recent studies that used transgenic mice harboring a 180-kD chromosome fragment *erythropoietin* transgene reported that *de novo* expression of α -smooth muscle antibody in interstitial fibroblasts correlates negatively with Epo expression, suggesting that additional superimposed intracellular programs render the Epo gene unresponsive to transcriptional stimuli (48,49). In this regard, differential expression of hypoxia-inducible factor (HIF) proteins, of globin transcription factor proteins, or hypermethylation of regulatory elements has been suggested to be involved (48,50). Relevance of such knowledge lies in possible use of these pathways to rescue endogenous Epo production in anemic patients (51).

Impaired Epo Production as Cause of Anemia

Anemia is a common complication of CKD, and the striking effectiveness of recombinant Epo proves that impaired production of endogenous Epo is a major cause of this (52). Decreased Epo levels in patients with CKD are obvious only when anemia is present but cannot be adequately elevated when hematocrit drops, suggesting that part of the Epo production capacity (*i.e.*, through loss of Epo-producing cells) is irreversibly lost (53–55). In general, decreased Epo levels (typically assessed by ELISA in serum) correlate with severity of decreased excretory kidney function. While Epo levels are better preserved in glomerular diseases than in interstitial diseases, suggesting that impaired Epo production is a direct consequence of interstitial disease (56), there is one exception as polycystic kidney disease is often associated with increased Epo production and polycythemia, due to aberrant HIF1 α accumulation around cysts due to local hypoxia (57). Nevertheless, circulating Epo levels have been suggested as diagnostic marker of the extent of tubulointerstitial involvement in

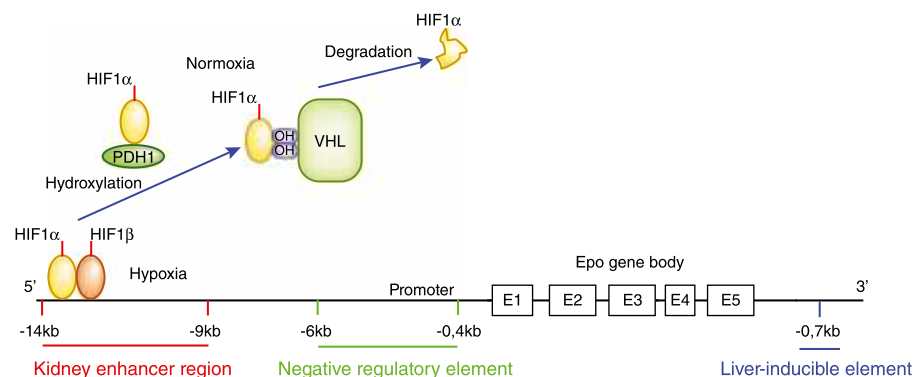


Figure 2. | Control of Epo expression. Renal Epo expression largely depends on enhancer regions located between -14 kb and -9.5 kb upstream of the Epo gene, whereas negative regulatory elements located between -6 and -0.4 kb play an important role in preventing ectopic Epo expression. Enhancer regions that control Epo expression in the liver are located at the 3' end at 0.7 kb downstream of the Epo locus. Under hypoxic conditions hypoxia-inducible factor (HIF)-1 α is stabilized, dimerizes with HIF-1 β , and translocates to the nucleus, where it can bind to hypoxia response elements within the kidney enhancer region and induce Epo transcriptional activity. Once normoxia is restored, prolyl hydroxylase (PDH1) hydroxylates HIF-1 α , causing its association with the von Hippel-Lindau tumor suppressor protein (VHL), ultimately causing ubiquitin-dependent degradation of HIF-1 α .

diabetic nephropathy (58), but due to superimposed regulating factors (including anemia, systemic inflammation, and reduced iron availability), utility of Epo levels as valid diagnostic marker for interstitial fibrosis could not be validated (53). Impaired Epo production as cause of anemia is not limited to CKD but is also increasingly being recognized as a cause of anemia in patients with diabetes mellitus. However, unlike in patients with CKD, Epo production responds adequately in diabetic patients to acute hypoxic stress, suggesting that in principle Epo-producing cells still exist, but that they don't sense the anemia appropriately (59). While in CKD interstitial cells appear to irreversibly lose capacity to produce Epo, it is conceivable that altered oxygen sensing plays a role in diabetes mellitus. In this regard, in early diabetes renal blood flow is increased, and it is plausible that resulting increased oxygen supply suppresses Epo production. In line with this, blockade of the renin-angiotensin system in rats increased interstitial blood flow (due to decreased intrarenal resistance) and anemia (60), possibly explaining the drop in hematocrit typically observed in the first month of therapy with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers in patients (61).

While supplementation with recombinant Epo is highly effective, it is associated with high costs, and its effectiveness can be impaired by antibody formation. Hence, stimulation of endogenous Epo production is an attractive therapeutic strategy. In this regard, inhibition of prolyl-hydroxylase (PHD; the enzyme that makes HIF accessible for Von Hippel-Lindau tumor suppressor protein [VHL] and subsequent proteolytic degradation) has emerged as the primary therapeutic target as several small molecule inhibitors have been developed. The rationale behind PHD inhibition is that conceptually it increases intracellular HIF levels and thus stimulates Epo production. In this regard, the PHD inhibitor FG-4592 is undergoing clinical testing in anemic patients with CKD stages 3 and 4. One possible drawback might be that currently available PHD inhibitors do not display PHD isoform specificity (among the known isoforms PHD1-3, PHD2 is considered the one involved in renal Epo

transcriptional control), and adverse effects of enhanced HIF activity (such as angiogenesis as the root of diabetic retinopathy) might be enhanced (62).

Adenosine

One often-underappreciated function of interstitial fibroblasts is their involvement in regulation of renal hemodynamics and microvascular function through generation of extracellular adenosine (63,64). To maintain glomerular filtration within a narrow range, the kidney responds to increased intratubular NaCl levels (upon increased GFR) by vasoconstriction of afferent arterioles (to decrease GFR) (65). A crucial mediator of this tubuloglomerular feedback loop is extracellular adenosine, which is generated through hydrolysis of ATP released by macula densa cells into the interstitium and which causes vasodilation of efferent arterioles (through activation of A2 adenosine receptors) and vasoconstriction of afferent arterioles through activation of A1 receptors, decreasing intraglomerular pressure and GFR (65). Furthermore, adenosine directly inhibits renin release in juxtaglomerular cells. Hence, when GFR and NaCl concentration of tubule fluid increase, adenosine is part of the tubuloglomerular feedback response to lower GFR and NaCl secretion; when GFR and NaCl concentrations decrease, adenosine levels drop under physiologic conditions (66). One enzyme that catalyzes hydrolysis of ATP to adenosine is 5'ectonucleotidase (5'NT, CD73), which is expressed in the kidney predominantly by resident fibroblasts (a characteristic that has made it a popular marker for renal fibroblasts) (63). 5'NT-deficient mice display substantial reduction of extracellular adenosine levels and reduced kidney weight; however, renal blood flow and GFR are unaltered (67), possibly because of intrinsic activity of interstitial ATP on vascular tone (64). In the chronically injured kidney, 5'NT-positive fibroblasts and extracellular adenosine levels accumulate, possibly contributing to the lowered GFR through afferent arteriole constriction, which is typical of CKD (2).

Renin

Renin is another protein that is released by renal cells and indirectly acts systemically (on BP), even though strictly speaking it is more an enzyme than a hormone. With more than 50,000 publications listed in PubMed to date, renin is among the most-studied proteins in biomedical sciences. Renin is a key regulator of the renin-angiotensin-aldosterone system, and plasma renin levels reflect the overall activity of this system (68). The circulating active form of renin cleaves angiotensinogen to form angiotensin I (69). Because angiotensinogen is highly abundant (its concentration is 1000-fold higher than that of angiotensin I), it is the plasma renin activity that determines the rate of angiotensin I formation (68).

Circulating renin is secreted by juxtaglomerular cells (also called granular cells or JGA cells), unique round cells of epithelioid appearance that contain myofilaments and abundant peroxisomes (70). While under physiologic conditions juxtaglomerular cells are located just at the outer edge of the renal interstitium (in the juxtaglomerular interstitium, at the walls of afferent arterioles just at the entrance into glomeruli) additional renin-producing cells are recruited extramurally within the interstitium (discussed in more detail below), and hence they are discussed here as “interstitial cells” for practical purposes (15). Release of renin by juxtaglomerular cells is regulated by hormones such as atrial natriuretic peptide and angiotensin II and by local factors contributed by adjacent tubular epithelial cells, by vascular smooth muscle cells and endothelial cells from afferent arterioles and by sympathetic nerve endings (71).

With regard to local regulatory factors, specialized tubular cells of the cortical thick ascending limb of the loop of Henle act together with macula densa cells to directly translate changes in the tubular NaCl concentration into inverse changes in renin secretion (72). In this system, macula densa cells sense intratubular NaCl concentrations through activity of the Na⁺-K-2Cl[−] cotransporter NKCC2 (BSC1) and respond to lower NaCl concentrations by release of prostanoids (generation of active prostaglandin E₂ and prostacyclin depends on cyclooxygenases) (71).

Furthermore, renin release is modulated by vascular smooth muscle cells and endothelial cells from afferent arterioles (71). In this system, renin stimulatory factors such as nitric oxide (formed by endothelial nitric oxide synthase) and prostacyclin/prostaglandin E₂ are formed in the endothelium adjacent to the granular cells, and inhibitory adenosine (which is converted into ATP) is released by vascular smooth muscle cells (73).

The sympathetic input provided by local nerve endings (in addition to circulating catecholamines) stimulate renin release through the β -adrenergic system (73). The magnitude of sympathetic control of renin release is revealed by double-knockout mice deficient in β 1- and β 2-receptors, which have 85% reduced plasma renin levels compared with wild-type control mice (74). Hence, renal denervation and baroreceptor stimulation conceptually seemed to be sound antihypertensive strategies. In this regard, failure of renal denervation to significantly lower BP in clinical studies is not yet entirely understood (75). Renal denervation also removes the input of the sympathetic system mediated by α -receptors, including hemodynamic and tubular effects, and systemic catecholamines remain fully functional; these findings suggest that baroreceptor stimulation might be the most promising approach to target the sympathetic nerve system to lower BP (76,77).

Renin secretion is further controlled by various hormones, autocrine, and other factors, including angiotensin II, as atrial natriuretic peptide, vasopressin, oxytocin, aldosterone, glucocorticoids, thyroid hormones, sex steroids, adipokines, dopamine, bradykinin, adrenomedullin, and neuropeptide Y (for further review, see extensive review elsewhere [71,73]). While availability of potent ACE inhibitors and angiotensin receptor blockers (and absence of potent renin blockers) long overshadowed the role of renin in clinical practice, understanding of direct renin-receptor mediated effects and availability of aliskiren revived interest in renin biology (78).

Under physiologic conditions, secretion and activation of prorenin by juxtaglomerular cells are sufficient for maintenance of GFR (71). During pathologic conditions, however, additional perivascular fibroblasts (*i.e.*, pericytes, vascular smooth muscle cells) of afferent arterioles express prorenin, which has led to increased interest in the ontogeny of renin-producing cells (79). Under basal conditions, renin produced by juxtaglomerular cells is sufficient to main BP and intravascular volume (71). Upon prolonged threat to volume homeostasis (*i.e.*, through exposing mice to a low salt diet and ACE inhibitors) additional cells along afferent arterioles express renin, in angiotensinogen-deficient mice renin is even expressed by fibroblasts (pericytes) all through the kidney cortex (80,81). Models of CKD are also associated with recruitment of additional renin-producing cells. Because increased renin expression is often at the root of hypertension, and because higher on-treatment plasma renin activity contributes to progression of CKD and increased cardiovascular risk, mechanisms that cause such inopportune increase in renin release and particularly recruitment of renin producers are of imminent interest (82,83).

Interplay of Renin-Angiotensin and Epo Systems

Clinicians know that patients with renovascular hypertension and kidney transplant recipients with transplant renal artery stenosis have higher hematocrit values compared with patients who have normal renal arteries (84). The underlying pathomechanism of this clinical observation is that ensuing renal hypoperfusion activates the renin-angiotensin system (as described in detail above) and that angiotensin II stimulates erythropoiesis through stimulating Epo secretion and through its direct stimulating effect on erythropoiesis in the bone marrow (85). This mechanism explains why effective blockage of the renin-angiotensin system through ACE inhibitors and/or angiotensin receptor blockers is often associated with a decrease in hematocrit (86,87) and that ACE inhibitors are effective in normalizing post-transplant erythrocytosis (88). ACE inhibitors even affect hematocrit in hemodialysis patients, who require recombinant Epo substitution because of impaired endogenous Epo production (as discussed in more detail above). This occurs because treatment with ACE inhibitors is associated with higher recombinant human Epo (rhEpo) requirements (whereas withdrawal of ACE inhibitors decreases rhEpo doses), reflecting the direct effect of angiotensin II on erythropoiesis (89). Exceptions to the link of renin-angiotensin system activation and increased hematocrit are often cases in which increment of red blood cells mass are masked by volume expansion (*i.e.*, in patients with severe heart failure) (90).

Hypertension is the most relevant side-effect of rhEpo therapy (91). However, the increase in BP that is often observed in dialysis patients receiving rhEpo therapy is independent of the renin-angiotensin system; several clinical studies did not demonstrate renin-angiotensin system activation upon rhEpo therapy (92). Instead, the increased BP observed with rhEpo therapy is most likely a direct systemic vasoconstriction (91). Nevertheless, rhEpo-induced hypertension is correctable with the typical antihypertensive therapeutic regimen, including ACE/angiotensin receptor blocker inhibition (91).

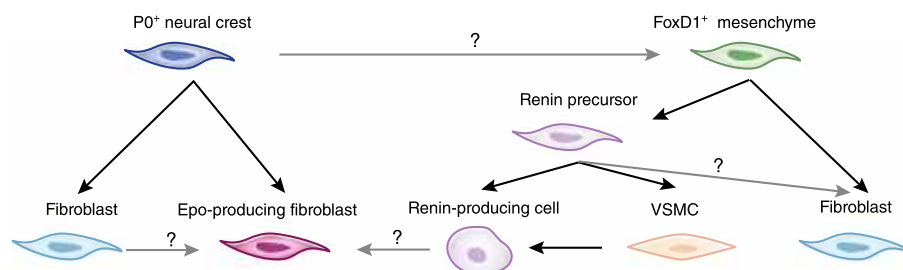
Origins of Hormone-Producing Interstitial Cells

If we are going to develop specific therapeutic interventions, we need to understand the origins and development of fibroblast subpopulations, including those that produce Epo, those that produce renin, and those that proliferate and lay down the extracellular matrix in fibrosis. Such studies are done by crossing mice in which cre-recombinase is expressed under control of promoters specific for a certain lineage (such as myelin protein zero for neural crest or FoxD1 for mesenchyme) or indicating Epo or renin expression (epo-cre or renin-cre) to mice that harbor a floxed reporter gene, irreversibly tagging them even when expression of the original marker is lost upon further cell differentiation.

Published data mostly suggest that renin- and Epo-producing cells are of two distinct lineages: Renin-producing cells are considered to be derivatives of FoxD1 mesenchymal cell progenitor cells, whereas Epo-producing cells have been suggested to be of neural crest origin, although existing data to support this are limited (Figure 3). Evidence for neural crest origin of Epo-producing cells stems from one study that analyzed *P0-Cre;R26tdRFP;Epo-GFP* triple transgenic mice (49). In this system, Cre-recombinase expression is driven by the myelin protein zero promoter, which is expressed by neural crest cells in early embryonic development (93), tagging deriving cells through recombination of the RFP reporter allele (94). These mice were crossed with reporter mice in which GFP is fused to a 180-kD chromosomal fragment containing the mouse *Epo* coding region and all necessary regulatory elements and displays specific Epo expression within kidney interstitial cells (95). On the basis of the observation that >75% of *Epo-GFP*-positive cells were also positive for RFP, it was assumed that most Epo-producing cells are of neural crest origin (information on percentage of RFP-positive cells

that were also positive for GFP was not provided) (49). This study also analyzed kidneys of *P0-Cre;R26ECFP* double transgenic mice (in these mice, derivate cells of myelin protein zero-positive cells are tagged through expression of ECFP) and found that almost all interstitial cells in the adult kidney that expressed PDGF receptor- β and/or CD73 were also ECFP positive. The researchers concluded that almost all interstitial fibroblasts were of neural crest origin. In summary, this study suggested that Epo-producing interstitial cells are derivatives of neural crest, just like all interstitial fibroblasts (Figure 3). The authors do not indicate whether Epo-producing cells are a specific subpopulation of fibroblasts or whether all fibroblasts can potentially produce Epo (49). This study somewhat contradicts a school of thought suggesting that all resident interstitial fibroblasts in adult kidneys are derivatives of mesenchymal FoxD1-positive progenitor cells (27).

This thinking is based on a study that used *FoxD1-GFP-Cre;R26STOPlacZ* double transgenic mice and found that almost all interstitial cells in the adult kidney expressing PDGF receptor- β and/or CD73 were also LacZ positive. Those investigators concluded that almost all interstitial fibroblasts were derivatives of FoxD1-positive mesenchymal progenitor cells (albeit only 20% of fibroblasts were LacZ positive when inducible *FoxD1-CreER;R26STOPlacZ* transgenic mice were used) (27). Neither study conclusively determined whether FoxD1 mesenchymal progenitors are of extrarenal origin (Figure 3). In this context, there is ample evidence that juxtaglomerular renin-producing cells are progeny of renin-positive precursor cells (cells that also give rise to perivascular fibroblast-like cells, which can be recruited to produce renin when needed). This concept is mainly based on studies that analyzed kidneys of *Ren-1d-Cre;R26STOPlacZ* and *Ren-1d-Cre;Z/EG* double transgenic mice as well as in *Ren-1d-Cre;R26STOPlacZ ;ATGF-/-* composite mice (in these mice, angiotensinogen deficiency causes additional recruitment of renin-producing cells [96]), which documented common lineage for perivascular fibroblasts and renin-producing juxtaglomerular cells (70,79). These aforementioned renin precursor cells are presumably progeny of FoxD1-positive progenitor cells, but this information has been disclosed only as “unpublished data” (Figure 3) (97). A recent study by the Kurtz group demonstrated procollagen production by renin precursor cells, hinting at a common lineage of renin cells and non-renin-producing fibroblasts (98).



To explore the role of hypoxia as possible stimulus for renin production, Kurtz and coworkers ablated VHL in renin-producing cells (*Renin-1d-Cre;Vhl^{lox/lox}*) based on the concept that absence of VHL causes HIF accumulation (due to insufficient proteolytic degradation), mimicking hypoxic signaling. Unexpected findings were that renin-producing cells were decreased (it had been widely presumed that hypoxia stimulates renin production) and that VHL-deficient juxtaglomerular cells produced Epo instead of renin (99). Although it is unclear whether such clamping of HIF at abnormally high levels (and resulting Epo overproduction and renin suppression) ever happens under in non-genetically modified conditions, this observation raises several conceptual questions. For example, future studies should determine whether renin and Epo production are interchangeable functions of a specialized interstitial cell type, whether all renal fibroblasts have potential to produce Epo or renin, or, in a broader view, what renin- and Epo-producing cells truly are.

Pathophysiology of the Renal Interstitium

Just as the renal interstitium plays an integral role in physiologic kidney function, it is a central determinant of the fate of diseased kidneys. Chronic progressive kidney injury is unequivocally associated with both quantitative

and qualitative changes of the renal interstitium, which are commonly referred to as “interstitial fibrosis.” The fibrotic interstitium is no longer invisible because it substantially expands (although in normal kidneys the relative volume taken up by the interstitium is no higher than 5%–10%, it often occupies >60% of kidney tissue in a severely diseased kidney). Such expansion is predominantly caused by accumulation of extracellular matrix (ECM; fibers), ECM-producing fibroblasts, and mononuclear cells. While ECM-producing fibroblasts (and often also renin-producing cells) accumulate in the fibrosing interstitium, Epo-producing fibroblasts are lost, raising the question of whether Epo-producing fibroblasts convert into non-Epo-producing fibroblasts in CKD and whether renin-producing cells could be therapeutically converted into Epo producers (Figure 4). While numerous cell types, including resident non-hormone-producing fibroblasts, endothelial cell, epithelial cells, and bone marrow-derived fibrocytes contribute to accumulation of ECM-producing fibroblasts in kidney fibrosis (29), the contribution of hormone-producing cells to accumulation of activated fibroblasts in CKD is yet unknown. Of note, there is a glaring disconnect between groups of different research interests: Most studies to elucidate origins of fibroblasts in fibrotic kidneys, including from our groups, did not analyze possible Epo or renin expression of fibroblasts. Studies from groups with an interest in Epo- and/or renin-expressing cells

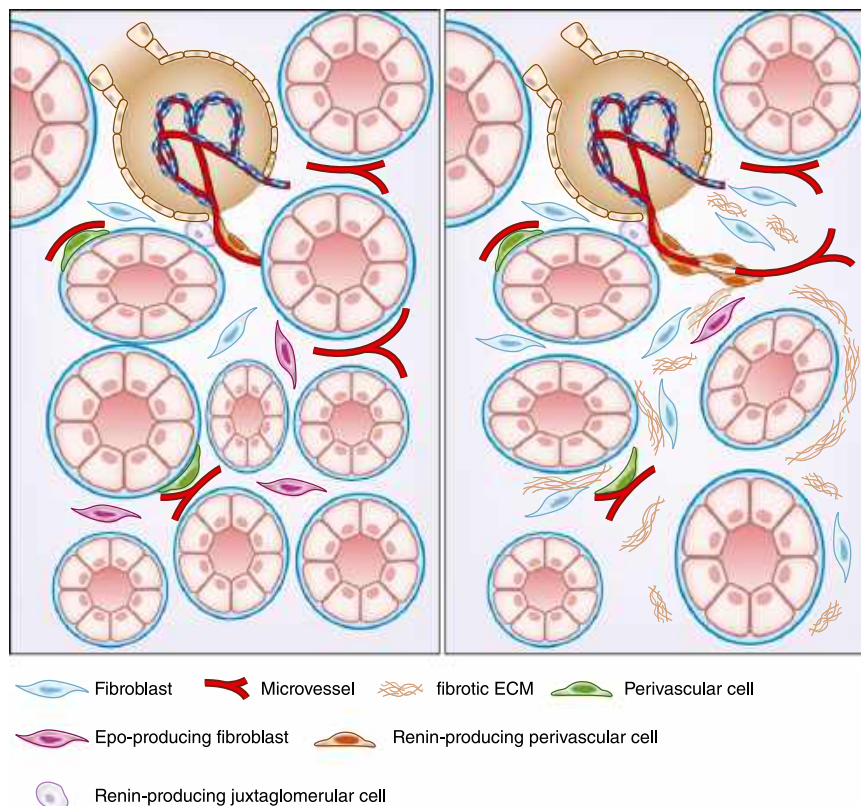


Figure 4. | From physiology to pathophysiology of the renal interstitium. Pathologic involvement of the interstitium, so-called fibrosis, determines progression of CKD. The schematic illustrates quantitative and qualitative changes that define the switch from physiologic (left) to fibrotic (pathophysiologic) interstitium (right). The interstitium not only increases in volume due to accumulation of fibrotic extracellular matrix, but fibroblasts capable of producing collagen accumulate whereas Epo-producing fibroblasts are diminished. Renin-producing perivascular cells accumulate within the interstitium.

reported that these cells could contribute to collagen production in CKD but did not use transgenic fate mapping systems, which are established in the fibrosis field. Impaired hormone production, however, is not the cardinal feature of the fibrosed interstitium in progressive CKD, as excretory kidney function is also affected. In fact, the extent of tubulointerstitial fibrosis is the single best determinant for requirement of RRT, even in primary glomerular nephropathies (100–102) because the expansion of the interstitium, paired with the overall kidney contraction, causes functional nephron loss through ensuing hypoxia (caused by microvascular rarefaction and hindered diffusion of oxygen and nutrients), and stenosis of glomerulotubular necks and tubular segments through physical pressure (103–105) (for in-depth information regarding the molecular events which underlie renal fibrogenesis we refer to extensive reviews elsewhere [4,105–108]).

Outlook

Epo and renin production are two of the kidney's most prominent functions, and these functions are localized to the fibroblasts of the renal interstitium. Thus, understanding of fibroblast origins and phenotypic plasticity is as relevant as ever. Future work will reveal whether reprogramming of fibroblasts may not only circumvent progressive deterioration of excretory kidney function but also limit relative Epo deficiency and renin overproduction in the future.

Acknowledgments

M.Z. is supported by DFG grant ZE523/2-1. R.K. is supported by National Institutes of Health grant DK081576 and by the Cancer Prevention and Research Institute of Texas and the Cancer Metastasis Research Center.

Disclosures

None.

References

- Lemley KV, Kriz W: Anatomy of the renal interstitium. *Kidney Int* 39: 370–381, 1991
- Kaissling B, Le Hir M: The renal cortical interstitium: Morphological and functional aspects. *Histochem Cell Biol* 130: 247–262, 2008
- Kuncio GS, Neilson EG, Haverty T: Mechanisms of tubulointerstitial fibrosis. *Kidney Int* 39: 550–556, 1991
- Zeisberg M, Neilson EG: Mechanisms of tubulointerstitial fibrosis. *J Am Soc Nephrol* 21: 1819–1834, 2010
- Eddy AA: Molecular insights into renal interstitial fibrosis [editorial]. *J Am Soc Nephrol* 7: 2495–2508, 1996
- Kriz W, Napiwotzky P: Structural and functional aspects of the renal interstitium. *Contrib Nephrol* 16: 104–108, 1979
- Knepper MA, Danielson RA, Saidel GM, Post RS: Quantitative analysis of renal medullary anatomy in rats and rabbits. *Kidney Int* 12: 313–323, 1977
- Takahashi-Iwanaga H: The three-dimensional cytoarchitecture of the interstitial tissue in the rat kidney. *Cell Tissue Res* 264: 269–281, 1991
- Teteris SA, Engel DR, Kurts C: Homeostatic and pathogenic role of renal dendritic cells. *Kidney Int* 80: 139–145, 2011
- Nelson PJ, Rees AJ, Griffin MD, Hughes J, Kurts C, Duffield J: The renal mononuclear phagocytic system. *J Am Soc Nephrol* 23: 194–203, 2012
- Kalluri R, Zeisberg M: Fibroblasts in cancer. *Nat Rev Cancer* 6: 392–401, 2006
- Zeisberg M, Strutz F, Müller GA: Role of fibroblast activation in inducing interstitial fibrosis. *J Nephrol* 13[Suppl 3]: S111–S120, 2000
- Enge M, Bjarnegård M, Gerhardt H, Gustafsson E, Kalén M, Asker N, Hammes HP, Shani M, Fässler R, Betsholtz C: Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. *EMBO J* 21: 4307–4316, 2002
- Abramson A, Lindblom P, Betsholtz C: Endothelial and non-endothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J Clin Invest* 112: 1142–1151, 2003
- Kurtz A: Renin release: Sites, mechanisms, and control. *Annu Rev Physiol* 73: 377–399, 2011
- Kurtz A, Eckardt KU, Neumann R, Kaissling B, Le Hir M, Bauer C: Site of erythropoietin formation. *Contrib Nephrol* 76: 14–20, discussion 21–23, 1989
- Komuro T: Re-evaluation of fibroblasts and fibroblast-like cells. *Anat Embryol (Berl)* 182: 103–112, 1990
- Franke WW, Schmid E, Osborn E, Weber K: Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc Natl Acad Sci U S A* 75: 5034–5038, 1978
- Lazarides E, Balzer DR Jr: Specificity of desmin to avian and mammalian muscle cells. *Cell* 14: 429–438, 1978
- Tuszynski GP, Frank ED, Damsky CH, Buck CA, Warren L: The detection of smooth muscle desmin-like protein in BHK21/C13 fibroblasts. *J Biol Chem* 254: 6138–6143, 1979
- Gardner H, Kreidberg J, Koteliensky V, Jaenisch R: Deletion of integrin alpha 1 by homologous recombination permits normal murine development but gives rise to a specific deficit in cell adhesion. *Dev Biol* 175: 301–313, 1996
- Vogel W, Gish GD, Alves F, Pawson T: The discoidin domain receptor tyrosine kinases are activated by collagen. *Mol Cell* 1: 13–23, 1997
- Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG: Identification and characterization of a fibroblast marker: FSP1. *J Cell Biol* 130: 393–405, 1995
- Marxer-Meier A, Hegyi I, Löffing J, Kaissling B: Postnatal maturation of renal cortical peritubular fibroblasts in the rat. *Anat Embryol (Berl)* 197: 143–153, 1998
- Thompson LF, Ruedi JM, Glass A, Low MG, Lucas AH: Antibodies to 5'-nucleotidase (CD73), a glycosyl-phosphatidylinositol-anchored protein, cause human peripheral blood T cells to proliferate. *J Immunol* 143: 1815–1821, 1989
- Fellström B, Klareskog L, Heldin CH, Larsson E, Rönnstrand L, Terracio L, Tufveson G, Wahlberg J, Rubin K: Platelet-derived growth factor receptors in the kidney—upregulated expression in inflammation. *Kidney Int* 36: 1099–1102, 1989
- Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS: Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 176: 85–97, 2010
- Gabbiani G, Kapanci Y, Barazzoni P, Franke WW: Immunohistochemical identification of intermediate-sized filaments in human neoplastic cells. A diagnostic aid for the surgical pathologist. *Am J Pathol* 104: 206–216, 1981
- LeBleu VS, Taduri G, O'Connell J, Teng Y, Cooke VG, Woda C, Sugimoto H, Kalluri R: Origin and function of myofibroblasts in kidney fibrosis. *Nat Med* 19: 1047–1053, 2013
- Lacombe C, Mayeux P: Biology of erythropoietin. *Haematologica* 83: 724–732, 1998
- Jelkmann W: Physiology and pharmacology of erythropoietin. *Transfus Med Hemother* 40: 302–309, 2013
- Fried W, Kilbridge T, Krantz S, McDonald TP, Lange RD: Studies on extrarenal erythropoietin. *J Lab Clin Med* 73: 244–248, 1969
- Cahan C, Hoekje PL, Goldwasser E, Decker MJ, Strohl KP: Assessing the characteristic between length of hypoxic exposure and serum erythropoietin levels. *Am J Physiol* 258: R1016–R1021, 1990
- Nangaku M, Eckardt KU: Hypoxia and the HIF system in kidney disease. *J Mol Med (Berl)* 85: 1325–1330, 2007
- Weidemann A, Johnson RS: Nonrenal regulation of EPO synthesis. *Kidney Int* 75: 682–688, 2009
- Chiang CK, Nangaku M, Tanaka T, Iwakawa T, Inagi R: Endoplasmic reticulum stress signal impairs erythropoietin production: a role for ATF4. *Am J Physiol Cell Physiol* 304: C342–C353, 2013

37. Miescher F: Über die Beziehungen zwischen Meereshöhe und Beschaffenheit des Blutes. *Korrespbl Schweiz Arz* 23: 809–830, 1893
38. Erslev A: Humoral regulation of red cell production. *Blood* 8: 349–357, 1953
39. Wojchowski D: Eugene Goldwasser (1922–2010). *Nature* 470: 40, 2011
40. Eckardt KU, Koury ST, Tan CC, Schuster SJ, Kaissling B, Ratcliffe PJ, Kurtz A: Distribution of erythropoietin producing cells in rat kidneys during hypoxic hypoxia. *Kidney Int* 43: 815–823, 1993
41. Pan X, Suzuki N, Hirano I, Yamazaki S, Minegishi N, Yamamoto M: Isolation and characterization of renal erythropoietin-producing cells from genetically produced anemia mice. *PLoS ONE* 6: e25839, 2011
42. Maxwell AP, Lappin TR, Johnston CF, Bridges JM, McGeown MG: Erythropoietin production in kidney tubular cells. *Br J Haematol* 74: 535–539, 1990
43. Unger EF, Thompson AM, Blank MJ, Temple R: Erythropoiesis-stimulating agents—time for a reevaluation. *N Engl J Med* 362: 189–192, 2010
44. Macdougall IC: Optimizing the use of erythropoietic agents—pharmacokinetic and pharmacodynamic considerations. *Nephrol Dial Transplant* 17[Suppl 5]: 66–70, 2002
45. Pagel H, Jelkmann W, Weiss C: O₂-supply to the kidneys and the production of erythropoietin. *Respir Physiol* 77: 111–117, 1989
46. Jelkmann W: Regulation of erythropoietin production. *J Physiol* 589: 1251–1258, 2011
47. Singh AK: Anemia of chronic kidney disease. *Clin J Am Soc Nephrol* 3: 3–6, 2008
48. Dewi FR, Fatchiyah F: Methylation impact analysis of erythropoietin (EPO) gene to hypoxia inducible factor-1 α (HIF-1 α) activity. *Bioinformation* 9: 782–787, 2013
49. Asada N, Takase M, Nakamura J, Oguchi A, Asada M, Suzuki N, Yamamura K, Nagoshi N, Shibata S, Rao TN, Fehling HJ, Fukatsu A, Minegishi N, Kita T, Kimura T, Okano H, Yamamoto M, Yanagita M: Dysfunction of fibroblasts of extrarenal origin underlies renal fibrosis and renal anemia in mice. *J Clin Invest* 121: 3981–3990, 2011
50. Souma T, Yamazaki S, Moriguchi T, Suzuki N, Hirano I, Pan X, Minegishi N, Abe M, Kiyomoto H, Ito S, Yamamoto M: Plasticity of renal erythropoietin-producing cells governs fibrosis. *J Am Soc Nephrol* 24: 1599–1616, 2013
51. Bernhardt WM, Wiesener MS, Scigalla P, Chou J, Schmieder RE, Günzler V, Eckardt KU: Inhibition of prolyl hydroxylases increases erythropoietin production in ESRD. *J Am Soc Nephrol* 21: 2151–2156, 2010
52. Artunc F, Risler T: Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease. *Nephrol Dial Transplant* 22: 2900–2908, 2007
53. Mercadal L, Metzger M, Casadevall N, Haymann JP, Karras A, Boffa JJ, Flamant M, Vrtovsnik F, Stengel B, Froissart M; NephroTest Study Group: Timing and determinants of erythropoietin deficiency in chronic kidney disease. *Clin J Am Soc Nephrol* 7: 35–42, 2012
54. Chandra M, Clemons GK, McVicar MI: Relation of serum erythropoietin levels to renal excretory function: Evidence for lowered set point for erythropoietin production in chronic renal failure. *J Pediatr* 113: 1015–1021, 1988
55. Thomas M, Tsalamandris C, Maclsaac R, Jerums G: Anaemia in diabetes: An emerging complication of microvascular disease. *Curr Diabetes Rev* 1: 107–126, 2005
56. de Klerk G, Wilimink JM, Rosengarten PC, Vet RJ, Goudsmit R: Serum erythropoietin (ESF) titers in anemia of chronic renal failure. *J Lab Clin Med* 100: 720–734, 1982
57. Eckardt KU, Möllmann M, Neumann R, Brunkhorst R, Burger HU, Lonnemann G, Scholz H, Keusch G, Buchholz B, Frei U: Erythropoietin in polycystic kidneys. *J Clin Invest* 84: 1160–1166, 1989
58. Inomata S, Itoh M, Imai H, Sato T: Serum levels of erythropoietin as a novel marker reflecting the severity of diabetic nephropathy. *Nephron* 75: 426–430, 1997
59. Bosman DR, Osborne CA, Marsden JT, Macdougall IC, Gardner WN, Watkins PJ: Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non-diabetic chronic renal failure. *Diabet Med* 19: 65–69, 2002
60. Naeshiro I, Sato K, Chatani F, Sato S: Possible mechanism for the anemia induced by candesartan cilexetil (TCV-116), an angiotensin II receptor antagonist, in rats. *Eur J Pharmacol* 354: 179–187, 1998
61. Ertürk S, Ateş K, Duman N, Karatan O, Erbay B, Ertuğ E: Unresponsiveness to recombinant human erythropoietin in haemodialysis patients: Possible implications of angiotensin-converting enzyme inhibitors. *Nephrol Dial Transplant* 11: 396–397, 1996
62. Franke K, Gassmann M, Wielockx B: Erythrocytosis: The HIF pathway in control. *Blood* 122: 1122–1128, 2013
63. Le Hir M, Kaissling B: Distribution and regulation of renal ecto-5'-nucleotidase: Implications for physiological functions of adenosine. *Am J Physiol* 264: F377–F387, 1993
64. Nishiyama A, Rahman M, Inscho EW: Role of interstitial ATP and adenosine in the regulation of renal hemodynamics and microvascular function. *Hypertens Res* 27: 791–804, 2004
65. Li L, Mizel D, Huang Y, Eisner C, Hoerl M, Thiel M, Schnermann J: Tubuloglomerular feedback and renal function in mice with targeted deletion of the type 1 equilibrative nucleoside transporter. *Am J Physiol Renal Physiol* 304: F382–F389, 2013
66. Vallon V, Mühlbauer B, Osswald H: Adenosine and kidney function. *Physiol Rev* 86: 901–940, 2006
67. Özüyan B, Ding Z, Buchheiser A, Koszalka P, Braun N, Gödecke A, Decking UK, Zimmermann H, Schrader J: Adenosine produced via the CD73/ecto-5'-nucleotidase pathway has no impact on erythropoietin production but is associated with reduced kidney weight. *Pflugers Arch* 452: 324–331, 2006
68. Kobori H, Nangaku M, Navar LG, Nishiyama A: The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev* 59: 251–287, 2007
69. Baylis C, Engels K, Hymel A, Navar LG: Plasma renin activity and metabolic clearance rate of angiotensin II in the unstressed aging rat. *Mech Ageing Dev* 97: 163–172, 1997
70. Sequeira Lopez ML, Pentz ES, Robert B, Abrahamson DR, Gomez RA: Embryonic origin and lineage of juxtaglomerular cells. *Am J Physiol Renal Physiol* 281: F345–F356, 2001
71. Castrop H, Höcherl K, Kurtz A, Schweda F, Todorov V, Wagner C: Physiology of kidney renin. *Physiol Rev* 90: 607–673, 2010
72. Skøtt O, Briggs JP: Direct demonstration of macula densa-mediated renin secretion. *Science* 237: 1618–1620, 1987
73. Hackenthal E, Paul M, Ganten D, Taugner R: Morphology, physiology, and molecular biology of renin secretion. *Physiol Rev* 70: 1067–1116, 1990
74. Kim SM, Chen L, Faulhaber-Walter R, Oppermann M, Huang Y, Mizel D, Briggs JP, Schnermann J: Regulation of renin secretion and expression in mice deficient in beta1- and beta2-adrenergic receptors. *Hypertension* 50: 103–109, 2007
75. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, Leon MB, Liu M, Mauri L, Negoita M, Cohen SA, Opail S, Rocha-Singh K, Townsend RR, Bakris GL; SYMPLICITY HTN-3 Investigators: A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 370: 1393–1401, 2014
76. Fisher JP, Paton JF: The sympathetic nervous system and blood pressure in humans: Implications for hypertension. *J Hum Hypertens* 26: 463–475, 2012
77. Davidson AC, Bisognano JD: Interventional approaches for resistant hypertension. *Curr Opin Nephrol Hypertens* 21: 475–480, 2012
78. Zeisberg M, Müller GA: Mechanistic insights into the anti-fibrotic activity of aliskiren in the kidney. *Hypertens Res* 35: 266–268, 2012
79. Sequeira Lopez ML, Pentz ES, Nomasa T, Smithies O, Gomez RA: Renin cells are precursors for multiple cell types that switch to the renin phenotype when homeostasis is threatened. *Dev Cell* 6: 719–728, 2004
80. Ayan S, Roth JA, Freeman MR, Bride SH, Peters CA: Partial ureteral obstruction dysregulates the renal renin-angiotensin system in the fetal sheep kidney. *Urology* 58: 301–306, 2001
81. Mimura I, Nangaku M: The suffocating kidney: Tubulointerstitial hypoxia in end-stage renal disease. *Nat Rev Nephrol* 6: 667–678, 2010

82. Sealey JE, Alderman MH, Furberg CD, Laragh JH: Renin-angiotensin system blockers may create more risk than reward for sodium-depleted cardiovascular patients with high plasma renin levels. *Am J Hypertens* 26: 727–738, 2013
83. Huby AC, Kavvadas P, Alfieri C, Abed A, Toubas J, Rastaldi MP, Dussaule JC, Chatziantoniou C, Chadjiachristos CE: The RenTg mice: A powerful tool to study renin-dependent chronic kidney disease. *PLoS ONE* 7: e52362, 2012
84. Tarazi RC, Frohlich ED, Dustan HP, Gifford RW Jr, Page IH: Hypertension and high hematocrit. Another clue to renal arterial disease. *Am J Cardiol* 18: 855–858, 1966
85. Vlahakos DV, Marathias KP, Madias NE: The role of the renin-angiotensin system in the regulation of erythropoiesis. *Am J Kidney Dis* 56: 558–565, 2010
86. Kuriyama S, Tomonari H, Tokudome G, Horiguchi M, Hayashi H, Kobayashi H, Ishikawa M, Hosoya T: Antiproteinuric effects of combined antihypertensive therapies in patients with overt type 2 diabetic nephropathy. *Hypertens Res* 25: 849–855, 2002
87. Jacobsen P, Andersen S, Jensen BR, Parving HH: Additive effect of ACE inhibition and angiotensin II receptor blockade in type I diabetic patients with diabetic nephropathy. *J Am Soc Nephrol* 14: 992–999, 2003
88. Vlahakos DV, Marathias KP, Agroyannis B, Madias NE: Post-transplant erythrocytosis. *Kidney Int* 63: 1187–1194, 2003
89. Ertürk S, Nergizoğlu G, Ateş K, Duman N, Erbay B, Karatan O, Ertuğ AE: The impact of withdrawing ACE inhibitors on erythropoietin responsiveness and left ventricular hypertrophy in haemodialysis patients. *Nephrol Dial Transplant* 14: 1912–1916, 1999
90. Volpe M, Tritto C, Testa U, Rao MA, Martucci R, Mirante A, Enea I, Russo R, Rubattu S, Condorelli GL, Cangianiello S, Trimarco B, Peschle C, Condorelli M: Blood levels of erythropoietin in congestive heart failure and correlation with clinical, hemodynamic, and hormonal profiles. *Am J Cardiol* 74: 468–473, 1994
91. Eckardt KU: Cardiovascular consequences of renal anaemia and erythropoietin therapy. *Nephrol Dial Transplant* 14: 1317–1323, 1999
92. Rosario R, Epstein M: Relationship between erythropoietin administration and alterations of renin-angiotensin-aldosterone. *J Renin Angiotensin Aldosterone Syst* 7: 135–138, 2006
93. Yamauchi Y, Abe K, Mantani A, Hitoshi Y, Suzuki M, Osuzu F, Kuratani S, Yamamura K: A novel transgenic technique that allows specific marking of the neural crest cell lineage in mice. *Dev Biol* 212: 191–203, 1999
94. Luche H, Weber O, Nageswara Rao T, Blum C, Fehling HJ: Faithful activation of an extra-bright red fluorescent protein in “knock-in” Cre-reporter mice ideally suited for lineage tracing studies. *Eur J Immunol* 37: 43–53, 2007
95. Obara N, Suzuki N, Kim K, Nagasawa T, Imagawa S, Yamamoto M: Repression via the GATA box is essential for tissue-specific erythropoietin gene expression. *Blood* 111: 5223–5232, 2008
96. Kim HS, Maeda N, Oh GT, Fernandez LG, Gomez RA, Smithies O: Homeostasis in mice with genetically decreased angiotensinogen is primarily by an increased number of renin-producing cells. *J Biol Chem* 274: 14210–14217, 1999
97. Sequeira Lopez ML, Gomez RA: Development of the renal arterioles. *J Am Soc Nephrol* 22: 2156–2165, 2011
98. Karger C, Kurtz F, Steppan D, Schwarzensteiner I, Machura K, Angel P, Banas B, Risteli J, Kurtz A: Procollagen I-expressing renin cell precursors. *Am J Physiol Renal Physiol* 305: F355–F361, 2013
99. Kurt B, Paliege A, Willam C, Schwarzensteiner I, Schuch K, Neymeyer H, Sequeira-Lopez ML, Bachmann S, Gomez RA, Eckardt KU, Kurtz A: Deletion of von Hippel-Lindau protein converts renin-producing cells into erythropoietin-producing cells. *J Am Soc Nephrol* 24: 433–444, 2013
100. Bohle A, Bader R, Grund KE, Mackensen S, Neunhoeffer J: Serum creatinine concentration and renal interstitial volume. Analysis of correlations in endocapillary (acute) glomerulonephritis and in moderately severe mesangioproliferative glomerulonephritis. *Virchows Arch A Pathol Anat Histol* 375: 87–96, 1977
101. Bohle A, Christ H, Grund KE, Mackensen S: The role of the interstitium of the renal cortex in renal disease. *Contrib Nephrol* 16: 109–114, 1979
102. Risdon RA, Sloper JC, De Wardener HE: Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 2: 363–366, 1968
103. Cohen EP: Fibrosis causes progressive kidney failure. *Med Hypotheses* 45: 459–462, 1995
104. Cohen EP, Regner K, Fish BL, Moulder JE: Stenotic glomerulotubular necks in radiation nephropathy. *J Pathol* 190: 484–488, 2000
105. Becker GJ, Hewitson TD: The role of tubulointerstitial injury in chronic renal failure. *Curr Opin Nephrol Hypertens* 9: 133–138, 2000
106. Zeisberg M, Kalluri R: Cellular mechanisms of tissue fibrosis. 1. Common and organ-specific mechanisms associated with tissue fibrosis. *Am J Physiol Cell Physiol* 304: C216–C225, 2013
107. Eddy AA: Progression in chronic kidney disease. *Adv Chronic Kidney Dis* 12: 353–365, 2005
108. Remuzzi G, Bertani T: Pathophysiology of progressive nephropathies. *N Engl J Med* 339: 1448–1456, 1998

Published online ahead of print. Publication date available at www.cjasn.org.



Handling of Drugs, Metabolites, and Uremic Toxins by Kidney Proximal Tubule Drug Transporters

Sanjay K. Nigam,^{*,†‡} Wei Wu,^{*} Kevin T. Bush,[†] Melanie P. Hoenig,[§] Roland C. Blantz,^{||¶} and Vibha Bhatnagar^{**}

Abstract

The proximal tubule of the kidney plays a crucial role in the renal handling of drugs (e.g., diuretics), uremic toxins (e.g., indoxyl sulfate), environmental toxins (e.g., mercury, aristolochic acid), metabolites (e.g., uric acid), dietary compounds, and signaling molecules. This process is dependent on many multispecific transporters of the solute carrier (SLC) superfamily, including organic anion transporter (OAT) and organic cation transporter (OCT) subfamilies, and the ATP-binding cassette (ABC) superfamily. We review the basic physiology of these SLC and ABC transporters, many of which are often called drug transporters. With an emphasis on OAT1 (SLC22A6), the closely related OAT3 (SLC22A8), and OCT2 (SLC22A2), we explore the implications of recent *in vitro*, *in vivo*, and clinical data pertinent to the kidney. The analysis of murine knockouts has revealed a key role for these transporters in the renal handling not only of drugs and toxins but also of gut microbiome products, as well as liver-derived phase 1 and phase 2 metabolites, including putative uremic toxins (among other molecules of metabolic and clinical importance). Functional activity of these transporters (and polymorphisms affecting it) plays a key role in drug handling and nephrotoxicity. These transporters may also play a role in remote sensing and signaling, as part of a versatile small molecule communication network operative throughout the body in normal and diseased states, such as AKI and CKD.

Clin J Am Soc Nephrol 10: 2039–2049, 2015. doi: 10.2215/CJN.02440314

The kidney proximal tubule is the site of elimination of a vast number of small molecules. These include drugs (e.g., antibiotics, antivirals, diuretics, nonsteroidal anti-inflammatory drugs, and antidiabetic agents), physiologically important metabolites (e.g., folate, α -ketoglutarate, urate, and carnitine), nutrients (e.g., vitamins and flavonoids), signaling molecules (e.g., odorants, cyclic nucleotides, and prostaglandins), exogenous toxins (e.g., mercurial conjugates and aristolochic acid), gut microbiome products (e.g., kynurenine), and endogenous toxins (so-called uremic toxins, such as indoxyl sulfate) (1–7). Apart from excreting unmodified small molecule drugs, the kidney handles many conjugated metabolites, most of which are produced by phase 1 and phase 2 metabolism in the liver (e.g., products of hydroxylation, sulfation, and glucuronidation reactions) (8). Genes for phase 1 and 2 reactions are also expressed in the kidney and are likely to be very important in metabolic functions of the proximal tubule cells of kidney as well (9), although this area of research is underexplored.

Consider a hypothetical hospitalized patient with stage 3 CKD, who may have slightly elevated levels of circulating uremic toxins (e.g., indoxyl sulfate) and is also being treated with β -lactam antibiotics, loop diuretics, statins, and antiviral agents. This scenario thus includes a host of drugs, metabolites, and molecules that are handled by proximal tubule transporters, which orchestrate their clearance from the blood and their elimination into the urine. The presence of gene variants of some of these transporters (possibly more

common given this patient's ethnic background) could cause differences in expression or function of the transporters in the proximal tubule of this patient compared with others on the ward. Furthermore, perhaps the patient's blood pH has varied considerably, thus altering the net charge of some of the aforementioned organic molecules, and thus their capacity to be transported into different body tissues and fluids or be eliminated. Many of these small molecules tend to be protein bound, and perhaps the patient's albumin concentration is low, which may further affect small molecule distribution and elimination.

While this patient is hypothetical, this type of scenario is not uncommon. The variables that affect serum, tissue, and body fluid levels of a single drug, toxin, or metabolite excreted by the transporters that handle small molecules is quite complicated; much more so if one simultaneously considers several small molecules. Nevertheless, a great deal of progress has been made in the past few decades on the basic biology of drug, toxin, and metabolite handling, including those functioning in the kidney proximal tubule. With these details at hand, integration of this information and application to clinical settings, such as the scenario presented in the preceding paragraph, should eventually be feasible.

Many of the small molecules of clinical interest are charged: organic anions, organic cations, or molecules that have a zwitterionic character (both positive and negative charges). Molecules that are too large or albumin bound have limited glomerular filtration, and excretion instead depends largely on tubular secretion. First,

^{*}Department of Medicine,

[†]Department of Pediatrics,

[‡]Department of Cell & Molecular Medicine,

^{||}Division of Nephrology-

Hypertension, and

^{**}Division of Family & Preventative

Medicine, University of California–San

Diego, La Jolla, California; [¶]Veterans

Affairs San Diego Healthcare System,

San Diego, California; and [§]Division of

Nephrology, Beth

Israel Deaconess Medical Center and

Harvard Medical School, Boston,

Massachusetts

Correspondence:

Dr. Sanjay K. Nigam, Department of

Pediatrics, Medicine (Nephrology) and

Cellular & Molecular Medicine, University

of California, San Diego, La Jolla, CA

92093-0693. Email:

snigam@ucsd.edu

the molecules flow through the peritubular capillaries, where they are extracted by multispecific drug transporters at the basolateral (blood) surface of the proximal tubule cell (Figure 1, A and B). For the most part, these molecules are secreted unchanged into the tubular lumen by a set of transporters at the apical (luminal or urine) surface of the proximal tubule cell. There appear to be more than two dozen types of transporters involved in the net transport of organic anion, organic cation, or organic zwitterions by the proximal tubule. In some cases (e.g., urate), the net transport may be the result of both secretion and absorption, but here we mostly focus on the role of proximal tubule transporters in net secretion in the setting of the normal kidney and in disease states.

Classification of Organic Ion Transporters

Organic ion transporters in the proximal tubule are frequently collectively called multispecific drug transporters because of their multispecific nature and their crucial role in drug handling. But depending on the discipline (physiology, biochemistry, or pharmacology), or for historical reasons, a single transporter can sometimes be described by multiple different names in the literature (Table 1) (1). This can make it confusing, even for researchers in the field. These multispecific transporters fall into two general families: solute carrier (SLC) or ATP-binding cassette (ABC) transporters (1,3,10,11). The SLCs generally transport substances either down their concentration gradient or against their concentration gradient coupled with movement of a second substance down its concentration gradient. In the kidney, the most important multispecific SLC transporters appear to be the organic anion transporters (OATs), including OAT1 (SLC22A6, originally described as novel kidney transporter [NKT]) and OAT3 (SLC22A8), which appear to be the main transporters inhibited by the drug probenecid, and the organic cation transporters (OCTs) such as OCT2 (SLC22A2) (1). But increasing attention is being paid to other members of multiple SLC families, including organic anion transporting polypeptides (OATP, or SLCO family), multidrug and toxin extrusion proteins (MATEs or SLC47 family), peptide transporters (SLC15 family), and organic carnitine/zwitterionic transporters (SLC22A4 and SLC22A5) (1,12,13).

ABC transporters use energy generated by the hydrolysis of ATP to transport molecules across cell membranes. Several ABC transporters, including P-glycoprotein (P-gp; ABCB1), also known as multidrug-resistant protein 1 (MDR1), and breast cancer resistance protein (BCRP, also known as ABCG2) play key roles in tubular efflux. Other key family members involved in kidney proximal tubule transport are the multidrug-associated resistance proteins, (MRP2 [also known as ABCC2] and MRP4 [ABCC4]), located on the apical (urinary) surface of the cell (1,10).

Most substrates of medical importance (e.g., drugs, metabolites, and toxins) are eliminated primarily by more than one renal transporter *in vivo*. Para-aminohippurate (PAH), however, is largely extracted from the blood *in vivo* by OAT1, the classic PAH transporter (14–16). Many common drugs (e.g., antivirals) are known to interact with more than one SLC and/or ABC transporter expressed in the kidney,

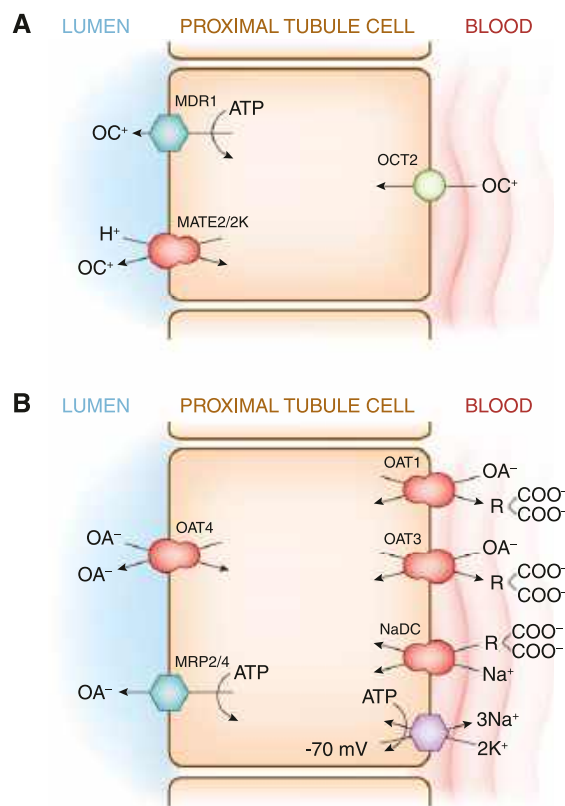


Figure 1. | Transcellular movement of organic cations (OCs) and organic anions (OAs) in kidney proximal tubule epithelial cells.

(A) Transcellular movement of OCs in a kidney proximal tubule epithelial cell. Movement of OCs are facilitated by the negative potential difference within the cell, which is maintained by the basolateral Na⁺,K⁺-ATPase (not shown). OCs enter the cell across the basolateral membrane by organic cation transporters (OCTs), such as OCT2. The authors note that OCT2 is also sometimes depicted as an OC exchanger. Secretion across the apical membrane into the lumen occurs by electroneutral exchange with H⁺ by solute carrier (SLC) transporters, including multidrug and toxin extrusion (MATE) protein 2/2K, and by ATP-binding cassette (ABC) transporters, such as multidrug-resistant protein 1 (MDR1), which use energy generated by ATP hydrolysis to transport molecules across the apical membrane. Only a fraction of the known transporters are shown. (B) OA transport *via* organic anion transporters (OATs) in a proximal tubule epithelial cell. Primary active transport of sodium out of the cell by the basolateral Na⁺,K⁺-ATPase creates the gradient that facilitates sodium dicarboxylate cotransporters (NaDCs) to move sodium and dicarboxylates [R(COO⁻)₂], such as α-ketoglutarate, into the cell. The resulting high intracellular concentration of dicarboxylates promotes uptake of OA across the basolateral membrane in exchange for [R(COO⁻)₂] by organic anion transporters (OAT1 and OAT3). Apical exit involves ABC transporters including multidrug-associated resistance proteins (MRP2, MRP4) and possibly other SLC transporters including OAT4 and urate transporter 1 (URAT1, not shown).

albeit with varying affinities and inhibitory constants (roughly 10 μM–1 mM); this makes it difficult to pin down the relative contributions of key transporters involved in renal elimination (1,17). Furthermore, while much of the data from *in vitro* transport assays and mouse knockout studies seems relevant to humans, caution must be exercised in extrapolating to human physiology.

Table 1. Select transporters involved in organic ion transport in the kidney proximal tubule

Name	HGNC Gene Symbol ^a	Common Symbol	Alternative Symbol(s)	Other Name
Organic Anion Transporter 1	<i>SLC22A6</i>	OAT1	NKT	Novel Kidney Transporter Reduced in Osteosclerosis Transporter
Organic Anion Transporter 3	<i>SLC22A8</i>	OAT3	ROCT	
Organic Anion Transporter 4	<i>SLC22A11</i>	OAT4	RST	Renal-Specific Transporter
Urate Anion Exchanger 1	<i>SLC22A12</i>	URAT1		
Organic Cation Transporter 2	<i>SLC22A2</i>	OCT2	P-gp	P-glycoprotein
Multidrug and Toxin Extrusion Protein 1	<i>SLC47A1</i>	MATE1		
Multidrug Resistance Protein 1	<i>ABCB1</i>	MDR1		
Breast Cancer Resistance Protein	<i>ABCG2</i>	BCRP		
Multidrug Resistance Associated Protein 2	<i>ABCC2</i>	MRP2		
Multidrug Resistance Associated Protein 4	<i>ABCC4</i>	MRP4		

^aFor each solute carrier (SLC), a number (the family series) is followed by the letter A, which serves as a divider, and then the number of the particular transporter within that family; ATP-binding cassette (ABC) nomenclature includes a letter (A–G) to describe the subfamily, and then the number of the transporter member. By convention, transporters are displayed as all uppercase letters when referring to proteins or human genes. HGNC, Human Genome Organisation gene nomenclature committee; OAT, organic anion transporter; URAT1, urate transporter 1; OCT, organic cation transporter; MATE1, multidrug and toxin extrusion protein 1; MDR1, multidrug-resistant protein 1; BCRP, breast cancer resistance protein; MRP, multidrug-associated resistance protein.

Basic Organic Ion Transporter Physiology

Excretion of organic cations begins with transport on the basolateral surface of the proximal tubular cell (Figure 1A). This is primarily achieved by OCT2, a transporter of organic cations, which takes advantage of the negative potential difference within the cell maintained by the basolateral Na^+/K^+ -ATPase. Several carriers on the apical surface subsequently transport organic cations across the apical membrane through electroneutral transport by exchange with proton (H^+), which capitalizes on the electrochemical gradient that favors movement of H^+ into the cells. The key apical transporters appear to be the MATEs (SLC47), but other SLC and ABC transporters may be involved.

OATs on the basolateral and apical membranes function in tandem to move organic anions from the blood, across the proximal tubule cells, and into the lumen (Figure 1B). The best-studied transporters on the basolateral membrane are OAT1 and OAT3, which have dozens of well established substrates. These transporters are organic anion/dicarboxylate exchangers, which use a tertiary active transport system on the basolateral side of the proximal tubule cell. The Na^+/K^+ -ATPase pumps sodium out of the cell, while sodium/dicarboxylate cotransporters move sodium and dicarboxylate molecules into the cell. The OAT antiporters move organic anions into the cell and dicarboxylate molecules out of the cell because the gradient favors outward movement of dicarboxylates, such as α -ketoglutarate, to the peritubular capillary. The current thinking is that MRP2 (ABCC2) and MRP4 (ABCC4) are the main apical efflux transporters of many of the organic anions taken up by OAT1 and OAT3, but other transporters, such as OAT4 and the urate transporter urate anion exchanger 1 (URAT1 [SLC22A12]), may also be involved (18). These transporters seem to work in concert to control the excretion of organic solutes (1–7,17).

Organic anion secretion has been recognized as an important function of the kidney for more than half a century.

In addition, it has long been known that secretion of organic anions by the kidney can be saturated, such that the addition of a second substance can inhibit secretion of the first. Since the mid-to-late 1990s, many of these transporters have been cloned (1,7,15,19,20) and a great deal of knowledge has accumulated as a result of transport studies in microinjected frog oocytes, transfected cells, and *in vivo* as well as *ex vivo* analysis of wild-type and knockout tissues (5,21,22). These data may help explain drug-drug interactions (DDIs) but also may explain differences in pharmacokinetics in different patients, some of whom may have transporter single-nucleotide polymorphisms (SNPs) that lead to more rapid or relatively delayed transport. This represents a large body of work by many investigators, and it is impossible to cover each transporter (1,7,21,23–26). In this review, however, we focus mainly on OAT1, OAT3, and OCT2, which have emerged as the primary transporters for many common drugs, toxins, and metabolites encountered in the clinical renal setting. To illustrate concepts, along the way we also highlight some interesting findings related to some of the other SLC and ABC transporters mentioned earlier. Additional details in the context of the broader field of drug transport can be found elsewhere (1).

Drugs and Toxins

There are now US Food and Drug Administration (FDA) regulatory guidelines to examine the transport of new drugs with a view toward understanding DDIs at the transporter level (27). Indeed, the FDA recommends that applications for new drugs and biologics in which renal secretion is significant be studied *in vitro* to determine whether these agents are substrates for OAT1, OAT3, or OCT2 (28). When these transporters appear to play a role, additional studies may be required. The list of drugs known to interact with these renal transporters is extensive, and there are data in human, rodents, and other species (5,17). This focus on drug

transporters promises to eventually improve understanding of pharmacokinetics in normal and diseased states (5).

The endogenous metabolite, creatinine, is a well described substrate for OCT2 and apical MATEs; competitive inhibition of these carriers can cause increases in serum creatinine by blocking its excretion (Figure 2A) (29–31). Drugs such as cimetidine and trimethoprim are examples. OATs, while mainly transporters of organic anions, can also transport some organic cations, including creatinine (32,33). In addition, newer agents, such as the integrase inhibitor dolutegravir and the novel pharmacoenhancer cobicistat, also block creatinine excretion (34,35). This effect appears to be largely from drug-metabolite interactions due to competition for OCTs, MATEs, and possibly OATs (29,32–35).

OCTs also transport organic cation drugs, such as metformin, and it has been postulated that polymorphisms in these transporters might account for the variability in clinical effect of this medication and the risk of toxicity. In the case of metformin, polymorphisms in OCT1, primarily expressed in hepatic cells, may explain variable efficacy, whereas polymorphisms in renal secretion initiated by OCT2 may contribute to variable pharmacokinetics (13).

The ABC transporter, P-gp (MDR1), which transports a wide range of molecules, is one of the best understood from the viewpoint of protein structure in relation to

transport function (1,36,37). P-gp has been widely studied in the context of cancer biology and its contribution to tumor resistance to chemotherapy (with increased expression of MDR1 on cancer cells associated with greater resistance). MDR1 also transports digoxin and interacts with quinidine.

OAT1, originally described as an NKT (15), and OAT3, also known in mice as reduced in osteosclerosis transporter (38), are the main transporters of organic anions in the proximal tubule (Figure 2B) (1,7). When the *Oat1* or *Oat3* genes are deleted in mice, the knockout mice have markedly blunted responses to loop and thiazide diuretics (14,39). These diuretics must enter the proximal tubule cell from the blood (basolateral) side and then be secreted into the proximal tubular lumen (apical side) before they can act more distally in the nephron to inhibit sodium transport. In addition, *Oat3* knockout mice are deficient in penicillin excretion (40), and both the *Oat1* and *Oat3* knockout kidney tissue has defective handling of antiviral agents, such as those used to treat HIV (41,42). It is worth noting that the kidney may handle medications in the same class differently. For example, furosemide is filtered and secreted, whereas filtration appears to be the main route of bumetanide entry into the tubular lumen (43,44); these pharmacologic differences may be important when diuretics are used in the setting of certain types of acute kidney disease and CKD.

Recent metabolomics data from *Oat3* knockout mice, together with *in vitro* studies, support the view that OAT3 is a major route of elimination for many compounds that undergo phase 2 modifications by drug-metabolizing enzymes in the liver (*e.g.*, glucuronidation) (8). Although detailed studies need to be performed in knockout mice, such modified compounds might be expected to include dietary plant products along with drugs and toxins. Many of these hepatically metabolized compounds are not routinely measured. In addition, it is possible that competition by drugs (*e.g.*, antibiotics, nonsteroidal anti-inflammatory drugs [NSAIDs], and antivirals) for OATs can affect the levels of many other metabolized compounds in the blood. Historically, probenecid was used to limit renal penicillin elimination where there was a critically small supply (45). It was later used to augment the effect of penicillin in the treatment of gonorrhea and other systemic infections (46). Probenecid is also used to block uric acid reabsorption in the proximal tubule for the treatment of gout (47); as discussed below, uric acid is a substrate of several proximal tubule drug transporters, including BCRP (ABCG2), OAT1, OAT3, and the related OAT family member (URAT1).

Drug transporters also handle environmental toxins, which may contribute to their toxicity to the renal tubules. For example, mercury exists in the blood in thiol conjugates (with glutathione and cystathione), which effectively act like organic anions. OAT1 and OAT3 appear to be the major transporters involved in elimination of mercuric conjugates, which can lead to renal and central nervous system toxicity following mercurial exposure (48–51). The proximal tubule of the kidney of the *Oat1* knockout mouse is largely resistant to nephrotoxic damage that occurs from systemic administration of mercuric chloride (Figure 3) (52). Aristolochic acid (53) and ochratoxin A (54), the putative nephrotoxins involved in Balkan endemic nephropathy, are both

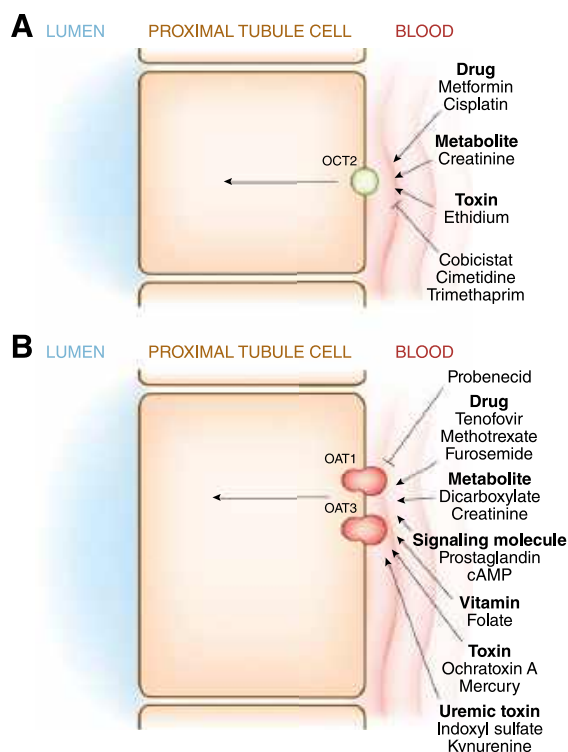


Figure 2. | Schematic of potential interactions between drugs, metabolites, and toxins due to competition for tubular secretion at the transporter level. (A) Some of the molecules that are transported by organic cation transporters (*e.g.*, OCT2). Transport is inhibited by cimetidine and trimethoprim and by the novel pharmacoenhancer, cobicistat. (B) Some of the substrates for the organic anion transporters OAT1 and/or OAT3. Transport is inhibited by probenecid.

transported into the proximal tubule by OATs. In this disorder, patients may present with evidence of tubular dysfunction or simply advanced CKD. The pathology is characterized by tubulointerstitial disease and fibrosis. Although the mechanism is not certain, these substances are clearly toxic to cultured renal epithelial cells (55). There is also a high incidence of uroepithelial cancer in these patients. Environmental toxins, such as the water-repellent polymer, perfluorooctanoic acid, are also substrates of OATs (56).

Uremic Toxins

Over 100 molecules have been implicated in the pathogenesis of uremic syndrome (57–60). Their precise roles are debated, but many of these are small organic anions, such as indoxyl sulfate, carboxy-methyl-propyl-furanpropionate, p-cresol sulfate, and kynurenine; molecules associated with CKD that can accumulate between dialysis sessions (57,59). These and many other potential uremic toxins are good OAT substrates. In *Oat1* knockout mice, some of these organic anions accumulate (61), although the mice do not appear ill and have normal life spans (14). Putative uremic toxins are also affected to varying degrees in *Oat3* knockout mice and *OATP4C1* transgenic rats (8,62,63). Uremic toxins interact with other SLC and ABC transporters as well. On the basis of *in vitro* transport data, the high levels of circulating organic anion

uremic toxins have the possibility of competing, at the level of transporters, for elimination and distribution of drugs, metabolites, and toxins (64). To the extent that transporters such as OAT1 and OAT3 play central roles in the regulation of systemic and local metabolism, this could contribute to the abnormalities in metabolism seen in uremia (1,7,65). How these transporters handle various uremic toxins in the kidney and nonrenal tissues in the setting of both normal physiology and disease is a fertile area for future translational research and may provide important insights into how to both delay and treat the symptoms of uremia.

Gut Microbiome Products, Nutrients, and Natural Products

There is growing evidence that many of the potential uremic toxins have their origin in the gut microbiome. Indole, for example, is produced by gut bacteria, undergoes sulfation in the liver, and is then excreted as indoxyl sulfate by the kidney (66). One of the primary renal transporters involved appears to be OAT1, which transports several gut microbial products or their metabolites (61,67). OAT1, OAT3, and other SLC and ABC transporters are also necessary for the elimination of dietary natural products, including a wide range of flavonoids, as well as vitamins, and thus might indirectly regulate vitamin-dependent metabolic pathways (8,67).

Metabolites and Signaling Molecules

Metabolomic studies in knockout animals, particularly *Oat1* and *Oat3* knockout mice, have confirmed a central role for drug transporters in the transport of many important metabolites and signaling molecules (8,14,61,67,68). These include α -ketoglutarate, which plays a central role in the Krebs cycle (tricarboxylic acid cycle); vitamins; molecules with antioxidant properties (*e.g.*, urate and flavonoids); and the gut microbial derivatives already described. Signaling molecules, such as cyclic nucleotides, prostaglandins, odorants, and conjugated steroids, are also eliminated *via* the OATs and other SLC and ABC drug transporters. Recent systems biology and omics integration of metabolomics and transcriptomics data from *Oat* knockout mice suggests that OATs and possibly all multispecific drug transporters play a role in the regulation of systemic and tissue metabolic and signaling processes (1,7,8,65,67,69). This type of information has led to the remote sensing and signaling hypothesis discussed below (65,69).

The transporter-mediated regulation of uric acid, mainly by renal transporters but also by nonrenal (*e.g.*, intestinal) transporters, appears quite complex in both humans and mice. Genome-wide association studies, *in vitro* transport data, and studies on knockout mice indicate that earlier models for uric acid handling were oversimplified. Several transporters have been implicated in renal urate handling to date; apical URAT1 (originally known as the renal-specific transporter in mice [70]) and SLC2A9 appear to play important roles in urate reabsorption, whereas apical ABCG2 (BCRP) and basolateral OAT1 and OAT3 are likely to play key roles in urate secretion (71,72). The complex regulation of uric acid may reflect a functional role that is

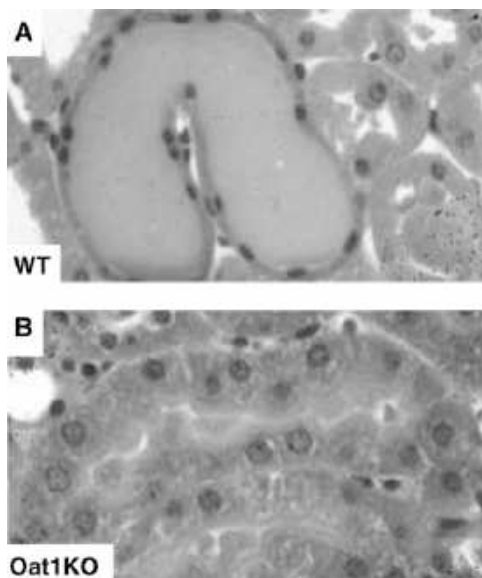


Figure 3. | Photomicrographs demonstrating tubular and cellular changes in wild-type (WT) and organic anion transporter 1 (*Oat1*) knockout kidneys upon exposure to mercuric chloride, HgCl_2 . (A) In the wild-type kidney, substantial tubular damage was seen, as indicated by the dilated tubular lumen and flattened tubule epithelial cells, after a single dose of HgCl_2 (4 mg/kg body wt, intraperitoneal). (B) In contrast, the *Oat1* knockout (*Oat1KO*) mice had normal tubules that appeared unaffected by mercury exposure. Although not shown, serum urea nitrogen levels increased significantly in the wild-type mouse but not in the knockout mouse with preserved tubules. (Adapted with permission from Torres *et al.* 52.)

not yet fully appreciated. Further study is clearly needed because uric acid has been implicated as both an antioxidant and a pro-oxidant and, in the case of the latter, a potential culprit in a range of disorders from atherosclerosis to hypertension and renal disease (73).

The details of transcriptional regulation of drug transporters in the proximal tubule are just beginning to unfold. Through a combination of systems-biology approaches involving analysis of transcriptomics and combining chromatin immunoprecipitation with DNA sequencing (ChIP-seq) data, it has been possible to implicate, and then show experimentally, that hepatocyte nuclear factor-4 α and hepatocyte nuclear factor-1 α are major regulators of OAT and OCT expression, as well as other proximal tubule transporters and drug-metabolizing enzymes (9,74,75). Unlike the extensive modern understanding of drug metabolism in the liver, the role of proximal tubule drug-metabolizing enzymes, in the context of both the proximal tubule cell and systemic physiology, is not well understood and is probably underappreciated. Future studies of the regulation of renal drug transporter expression by pharmaceuticals, uremic toxins, and environmental agents are likely to have a major effect on our understanding of the pathophysiology of CKD, as well as renal pharmacology and toxicology. It is conceivable that the transcription factors in the proximal tubule coordinate the regulation of drug transporter gene expression by sensing levels of signaling molecules, metabolites, drugs, and toxins, and responding by producing and deploying additional transporters as needed (1,7,65,69).

DDIs and Drug-Metabolite Interactions

Knowledge of DDIs at the level of organic ion transport in the kidney can also influence care. Some DDIs, such as penicillin and probenecid, are well established and have been put to clinical use; the half-life of penicillin is greatly prolonged by coadministration of probenecid, an OAT inhibitor (45–47). In contrast, some DDIs can lead to dire consequences. Methotrexate is taken up from the blood *via* OATs. NSAIDs can inhibit OATs, and when methotrexate and NSAIDs are used together, methotrexate toxicity can occur, manifesting as severe bone marrow suppression (76,77). DDIs at the level of P-glycoprotein (MDR1/ABCB1) in the proximal tubule and elsewhere are thought to explain the well known digoxin-quinidine interaction resulting in digoxin toxicity, including arrhythmias (78). Poor renal function can further complicate the clinical picture (79).

Whereas DDI *via* substrate competition has been well recognized (80), drug-metabolite interaction by a similar mechanism has received comparatively less attention. Considerable *in vitro* and substrate modeling (*e.g.*, pharmacophore) data suggest that drug-metabolite interaction could be a substantial problem because the drugs and metabolites handled by OATs and other drug transporters have structural similarities (61,81). This may be especially important in a setting such as CKD, in which circulating organic anion levels (*e.g.*, uremic toxins such as indoxyl sulfate) are high. With the broader application of metabolomics methods, this should become clearer in the near future.

Pharmacogenomic and Toxicogenomic Considerations

Early studies of transporter polymorphisms raised the possibility that multiple SNPs in basolateral (*e.g.*, OAT1 and OAT3) (82,83) and apical (*e.g.*, URAT1, OAT4, MRP2, and MRP4) drug transporters may affect the net transport of drugs, toxins, and metabolites from blood to urine (50,84). In addition, noncoding SNPs that regulate drug transporter expression might be particularly important (82,85). These polymorphisms may explain differences in drug response and toxicity among individuals. While emerging data seem to be consistent with these notions, there is much to be done in order to improve understanding of how coding or noncoding SNPs in renal drug transporter genes, and particularly combinations of these SNPs, affect overall renal handling of drugs, metabolites, and toxins (83).

As noted earlier, OAT1 and OAT3 are the major transporters of loop and thiazide diuretics, and secretion into the urinary space by the proximal tubular cells is necessary for these diuretics to induce the desired natriuresis by inhibiting sodium transport in later tubule segments. A noncoding region polymorphism has been identified in patients with diuretic resistance that could affect OAT1 and/or OAT3 expression (86). Polymorphisms in OAT1, OAT3, and MRP2 are more common among miners exhibiting toxicity from mercury-containing vapors (50). Additionally, OAT3 polymorphisms have been associated with altered cephalosporin handling (87), and suggestive evidence indicates the possible involvement of OATs in antiviral and methotrexate elimination (88,89). One of the better-studied examples of drug transporter polymorphisms affecting renal drug elimination is that of OCT2 (SLC22A2). Patients with certain SNPs in this transporter have altered metformin handling (90). In addition, polymorphisms in MATE1, on the apical membrane, may also affect metformin handling (91). OCT2 polymorphisms that affect transport function also may play a role in determining whether cisplatin nephrotoxicity occurs, as cisplatin gains entry into proximal tubule cells from the basolateral membrane (92).

Overall, results from *in vitro* transport studies in cells that overexpress transporters, together with results from studies in knockout animals (or tissues derived from them), and rat experiments (*e.g.*, after probenecid treatment) seem reasonably concordant with available human clinical data. Although there are sure to be many caveats and exceptions, this overall concordance is a very important point from an experimental and translational standpoint; it indicates that the considerable *in vitro*, *ex vivo*, and *in vivo* knockout mouse and rat data can continue to be used to help guide our clinical understanding.

Pediatric Developmental Pharmacology and Drug Elimination in the Aging Population

Most of our understanding of renal drug elimination comes from analysis of adult patients and adult animals. In addition, most studies have only limited consideration of ethnicity, sex, and the extremes of age (3,93). Human pediatric kidney data remain limited, so what we currently know, especially from a mechanistic standpoint, comes largely from a limited number of rodent studies. For example, it is known that drug transporter expression occurs

early in embryogenesis; indeed, several renal drug transporters are expressed transiently in the developing central nervous system and other tissues (94). Late in rodent gestation, the expression is largely limited to the future proximal tubules of the kidney (15,95); thereafter, there is a burst in renal expression around the time of birth, eventually reaching (and perhaps overshooting for a short time) adult expression (9,96). This is paralleled by functional changes, such as increasing PAH clearance (96). There is evidence in animals for a developmental inducibility window, during which renal drug transporters may, during the early postnatal period, be induced by substrates or by hormones (97,98). If this is true in humans, substrate induction could theoretically enhance the ability of a premature infant's kidney to excrete potentially deleterious drugs and toxins. A coordinated response by the postnatal kidneys with the liver is also needed to excrete drugs and metabolites during the continuing period of maturation (3). Furthermore, many OAT1 and OAT3 substrates are drugs, toxins, and metabolites that have been modified by phase 1 (e.g., cytochrome-dependent) or phase 2 (conjugation, such as glucuronidation) reactions. For example, OAT3 is responsible for the elimination of many glucuronidated compounds (8). How this liver-kidney coordination is achieved during postnatal maturation, or during recovery from organ injury, is not yet well understood.

Adverse drug reactions in the elderly are also a major clinical concern. Some of these adverse drug reactions may be partly related to altered expression or function of drug transporters in the aging kidney (99,100). However, data on this important issue are limited.

AKI and CKD

Following AKI from ischemia or toxins, substantial changes have been observed in transcript and protein expression of many drug transporters. Initially, expression of certain transporters seems to decrease, followed by upregulation during recovery (24). The extent to which this is reflected in functional handling of specific drugs, metabolites, and toxins is not well understood. As discussed earlier, many of the putative uremic toxins, including indoxyl sulfate and kynurenine, are excellent substrates of OATs and other drug transporters, such as OATPs, and they accumulate in the knockout or transgenic models of these transporters. These substances may themselves be toxic to the tubule cell and may also contribute to the progression of CKD (8,61–63,101).

Although drug transporters on the basolateral and apical membranes of the proximal tubule protect from systemic toxicity by enhancing drug and toxin elimination, they are, in some instances, the mechanism by which substances toxic to the proximal tubule gain entry. Cephaloridine, a first-generation cephalosporin that has largely been replaced by newer agents with better bioavailability and less nephrotoxicity, is one such example. Cephaloridine, like other cephalosporins, is excreted unchanged by renal tubular secretion. Yet this agent accumulates in the proximal tubule; after basolateral OAT3 uptake, secretion by apical membrane transporters may not be sufficiently rapid to avoid toxicity, perhaps because of the zwitterionic nature of this agent (102). Toxicity appears to be related to oxidative stress from depletion of reduced glutathione (103). This imbalance in

proximal tubular cell entry and exit has also been implicated in the proximal tubule defect produced by the antiviral agent tenofovir. Indeed, *Oat1* knockout mice appear protected from tenofovir-induced proximal tubular damage, whereas those with loss of apical efflux of tenofovir seen in *Mrp4* knockout mice were particularly susceptible (104).

The role of drug transporters in proximal tubule metabolism raises some interesting questions in the context of renal ischemia and other types of injury. The proximal tubule exhibits some unique metabolic characteristics (105). Although glycolytic enzymes are present in the proximal tubule, the cells are largely obligate oxidative in metabolic character; they are relatively incapable of glycolytic metabolism and poorly utilize glucose as a preferred substrate (77,106). Thus, in the setting of cell stress, the proximal tubule cells might be expected to depend more on gluconeogenesis, whereby glucose is synthesized from substrates such as lactate, a compound not produced by proximal tubules but potentially taken up by proximal tubule transporters. Kidney gluconeogenesis then differs from hepatic gluconeogenesis by using lactate as a primary substrate. Because proximal tubules are unable to shift to glycolytic activity, the proximal tubule cells are a potential victim of hypoxic damage in comparison to renal cells located more distally in the nephron, which retain considerable glycolytic capacity, in keeping with the often lower oxygen levels in the environment. The proximal tubule does require substrates and coupling OAT transport with α -ketoglutarate as the intracellular counter-transporting molecule, plays a role in regulating the intracellular levels of citric acid cycle molecules involved in oxidative metabolism. This also raises a question as to whether changes in net proximal tubule OAT activity as a result of altered expression levels or competing anions could affect proximal tubular metabolism and ability to maintain normal oxidative substrate supply when the proximal tubule dynamics are altered in the context of extremes of age, stress, injury, or recovery from injury. It may thus be very interesting to study whether resistance to hypoxia and ATP depletion can be conditioned by altering OAT function.

Remote Sensing and Signaling Hypothesis

In general, the application of multiple omics methods (e.g., metabolomics and transcriptomics in wild-type and knockout animals) combined with systems biology approaches to reconstruct drug transporter-dependent metabolism is beginning to yield a new perspective on the role of renal and nonrenal SLC and ABC multispecific drug transporters. Transport of molecules by various SLC and ABC drug transporters in and out of cells could play a role in remote communication between cells and tissues as well as interfacing body fluids. Because SLC and ABC drug transporters are selectively expressed in different cells (e.g., kidney, choroid plexus, intestine, biliary tract, liver, brain capillary endothelium, olfactory mucosa, placenta, mammary gland, and testes) that interface with body fluids that bathe other cells, these so-called drug transporters may be part of a remote sensing and signaling system (1,7,65,69) involved in cell, tissue, and organ crosstalk. These transporters are also well situated to play a role in mediating

and/or restoring homeostasis after injury; certain aspects of the pathophysiology of AKI and CKD may be considered disordered remote sensing and signaling. The broader remote sensing and signaling hypothesis has been explained in more detail in other articles (1,7,65,69).

Several OAT isoforms appear somewhat specialized for transport of classic signaling molecules and antioxidants and therefore well suited for signaling: liver-expressed OAT2 transports cyclic guanosine monophosphate (107), placental-expressed OAT4 transports conjugated estrogens (108), olfactory-expressed OAT6 has high selectivity for odorants (109), and OATPG seems specialized for prostaglandins (110). The drug transporters mediating the influx and efflux through tissues and body fluid compartments (e.g., blood, urine, bile, cerebrospinal fluid, and amniotic fluid) could regulate remote communication *via* transport of key (potentially rate-limiting) metabolites and signaling molecules, hence regulating the movement of these molecules across tissue barriers in the body and also at the cellular level. Various types of data suggest that these SLC and ABC drug transporters are also involved in communication between gut bacteria and the body, and also across the maternal-fetal and maternal-neonate barriers *via* breast milk (1, 7).

In addition to a potential contribution to signaling in their own right, various drug transporters are directly affected by signaling molecules, which may govern transporter density on the cellular membrane. Drug transporters, like other transporters in the cell, may traffic from an internal pool, which might be deployed as needed or internalized (111) under stimulation by signaling pathways. Internalization or abnormal trafficking may affect drug excretion.

Summary

The hypothetical clinical vignette presented at the outset of this article represents the challenges that can be seen in many patients. The ballooning data regarding handling of small molecule drugs, metabolites, nutrients, and toxins by multispecific transporters expressed in the proximal tubule still do not explain the complexity of organic ion transport at play in the patient. While the discussion here has tended to focus more on renal drug transporters of the SLC22 family (especially OAT1 and OAT3), it is important to emphasize that many of the same principles and considerations are likely to apply to other SLC and ABC drug transporters in the kidney. Moreover, by focusing on the differences in tissue expression patterns, substrate specificities, regulation in development, evolutionary biology, and disease states, the field is just beginning to understand the role these drug transporters play not only in drug and toxin handling but in normal physiology and pathophysiology (24,65,69,112,113). Viewing uremia partly as a disorder of remote sensing and signaling may lead to new avenues of research and, possibly, novel therapeutic approaches.

Acknowledgments

This work was supported by National Institutes of Health grants GM104098 and GM098449 to S.K.N.

Disclosures

None.

References

1. Nigam SK: What do drug transporters really do? *Nat Rev Drug Discov* 14: 29–44, 2015
2. Burckhardt G: Drug transport by Organic Anion Transporters (OATs). *Pharmacol Ther* 136: 106–130, 2012
3. Nigam SK, Bhatnagar V: How much do we know about drug handling by SLC and ABC drug transporters in children? *Clin Pharmacol Ther* 94: 27–29, 2013
4. Nigam SK, Bush KT, Bhatnagar V: Drug and toxicant handling by the OAT organic anion transporters in the kidney and other tissues. *Nat Clin Pract Nephrol* 3: 443–448, 2007
5. VanWert AL, Gionfriddo MR, Sweet DH: Organic anion transporters: Discovery, pharmacology, regulation and roles in pathophysiology. *Biopharm Drug Dispos* 31: 1–71, 2010
6. Ahn SY, Bhatnagar V: Update on the molecular physiology of organic anion transporters. *Curr Opin Nephrol Hypertens* 17: 499–505, 2008
7. Nigam SK, Bush KT, Martovetsky G, Ahn SY, Liu HC, Richard E, Bhatnagar V, Wu W: The organic anion transporter (OAT) family: A systems biology perspective. *Physiol Rev* 95: 83–123, 2015
8. Wu W, Jamshidi N, Eraly SA, Liu HC, Bush KT, Palsson BO, Nigam SK: Multispecific drug transporter Slc22a8 (Oat3) regulates multiple metabolic and signaling pathways. *Drug Metab Dispos* 41: 1825–1834, 2013
9. Martovetsky G, Tee JB, Nigam SK: Hepatocyte nuclear factors 4 α and 1 α regulate kidney developmental expression of drug-metabolizing enzymes and drug transporters. *Mol Pharmacol* 84: 808–823, 2013
10. Masereeuw R, Russel FG: Regulatory pathways for ATP-binding cassette transport proteins in kidney proximal tubules. *AAPS J* 14: 883–894, 2012
11. You G, Morris ME: Overview of drug transporter families. In: *Drug Transporters: Molecular Characterization and Role in Drug Disposition*, 2nd Ed., edited by You G, Morris ME, Hoboken, NJ, John Wiley & Sons, Inc., 2014, pp 1–6
12. Bush KT, Nagle M, Truong DM, Bhatnagar V, Kaler G, Eraly SA, Wu W, Nigam SK: Organic anion transporters. In: *Drug Transporters: Molecular Characterization and Role in Drug Disposition*, 2nd Ed., edited by You G, Morris ME, Hoboken, NJ, John Wiley & Sons, Inc., 2014, pp 25–42
13. Koepsell H: The SLC22 family with transporters of organic cations, anions and zwitterions. *Mol Aspects Med* 34: 413–435, 2013
14. Eraly SA, Vallon V, Vaughn DA, Gangoi JA, Richter K, Nagle M, Monte JC, Rieg T, Truong DM, Long JM, Barshop BA, Kaler G, Nigam SK: Decreased renal organic anion secretion and plasma accumulation of endogenous organic anions in OAT1 knock-out mice. *J Biol Chem* 281: 5072–5083, 2006
15. Lopez-Nieto CE, You G, Bush KT, Barros EJ, Beier DR, Nigam SK: Molecular cloning and characterization of NKT, a gene product related to the organic cation transporter family that is almost exclusively expressed in the kidney. *J Biol Chem* 272: 6471–6478, 1997
16. Sweet DH, Wolff NA, Pritchard JB: Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. *J Biol Chem* 272: 30088–30095, 1997
17. Morrissey KM, Stocker SL, Wittwer MB, Xu L, Giacomini KM: Renal transporters in drug development. *Annu Rev Pharmacol Toxicol* 53: 503–529, 2013
18. Miyazaki H, Anzai N, Ekaratanawong S, Sakata T, Shin HJ, Jutabha P, Hirata T, He X, Nonoguchi H, Tomita K, Kanai Y, Endou H: Modulation of renal apical organic anion transporter 4 function by two PDZ domain-containing proteins. *J Am Soc Nephrol* 16: 3498–3506, 2005
19. Gründemann D, Gorboulev V, Gambaryan S, Veyhl M, Koepsell H: Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* 372: 549–552, 1994
20. Simonson GD, Vincent AC, Roberg KJ, Huang Y, Iwanij V: Molecular cloning and characterization of a novel liver-specific transport protein. *J Cell Sci* 107: 1065–1072, 1994
21. Burckhardt G, Burckhardt BC: In vitro and in vivo evidence of the importance of organic anion transporters (OATs) in drug therapy. In: *Drug Transporters*, edited by Fromm MF, Kim RB, Berlin, Heidelberg, Springer, 2011, pp 29–104

22. You G: Membrane transporters in drug disposition. *Pharm Res* 25: 441–443, 2008
23. Eraly SA, Bush KT, Sampogna RV, Bhatnagar V, Nigam SK: The molecular pharmacology of organic anion transporters: From DNA to FDA? *Mol Pharmacol* 65: 479–487, 2004
24. Saito H: Pathophysiological regulation of renal SLC22A organic ion transporters in acute kidney injury: Pharmacological and toxicological implications. *Pharmacol Ther* 125: 79–91, 2010
25. Sweet DH, Bush KT, Nigam SK: The organic anion transporter family: From physiology to ontogeny and the clinic. *Am J Physiol Renal Physiol* 281: F197–F205, 2001
26. Wang L, Sweet DH: Renal organic anion transporters (SLC22 family): Expression, regulation, roles in toxicity, and impact on injury and disease. *AAPS J* 15: 53–69, 2013
27. Lee SC, Zhang L, Huang SM: Regulatory science perspectives on transporter studies in drug development. In: *Drug Transporters: Molecular Characterization and Role in Drug Disposition*, 2nd Ed, edited by You G, Morris ME, Hoboken, NJ, John Wiley & Sons, 2014, pp 473–487
28. U.S. Food and Drug Administration: *Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. Rockville, MD, U.S. Food and Drug Administration, Drug-Drug Interaction Working Group, Center for Drug Evaluation and Research (CDER), 2012, pp 1–75
29. Ciarimboli G, Lancaster CS, Schlatter E, Franke RM, Sprowl JA, Pavenstädt H, Massmann V, Guckel D, Mathijssen RH, Yang W, Pui CH, Relling MV, Herrmann E, Sparreboom A: Proximal tubular secretion of creatinine by organic cation transporter OCT2 in cancer patients. *Clin Cancer Res* 18: 1101–1108, 2012
30. Imamura Y, Murayama N, Okudaira N, Kurihara A, Okazaki O, Izumi T, Inoue K, Yuasa H, Kusuhara H, Sugiyama Y: Prediction of fluoroquinolone-induced elevation in serum creatinine levels: A case of drug-endogenous substance interaction involving the inhibition of renal secretion. *Clin Pharmacol Ther* 89: 81–88, 2011
31. Motohashi H, Inui K: Multidrug and toxin extrusion family SLC47: Physiological, pharmacokinetic and toxicokinetic importance of MATE1 and MATE2-K. *Mol Aspects Med* 34: 661–668, 2013
32. Ahn SY, Eraly SA, Tsigelny I, Nigam SK: Interaction of organic cations with organic anion transporters. *J Biol Chem* 284: 31422–31430, 2009
33. Vallon V, Eraly SA, Rao SR, Gerasimova M, Rose M, Nagle M, Anzai N, Smith T, Sharma K, Nigam SK, Rieg T: A role for the organic anion transporter OAT3 in renal creatinine secretion in mice. *Am J Physiol Renal Physiol* 302: F1293–F1299, 2012
34. Lepist EI, Zhang X, Hao J, Huang J, Kosaka A, Birkus G, Murray BP, Bannister R, Cihlar T, Huang Y, Ray AS: Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat. *Kidney Int* 86: 350–357, 2014
35. Reese MJ, Savina PM, Generaux GT, Tracey H, Humphreys JE, Kanaoka E, Webster LO, Harmon KA, Clarke JD, Polli JW: In vitro investigations into the roles of drug transporters and metabolizing enzymes in the disposition and drug interactions of dolutegravir, a HIV integrase inhibitor. *Drug Metab Dispos* 41: 353–361, 2013
36. Vlaming ML, Teunissen SF, van de Steeg E, van Esch A, Wagenaar E, Brunsvelde L, de Greef TF, Rosing H, Schellens JH, Beijnen JH, Schinkel AH: Bcrp1;Mdr1a/b;Mrp2 combination knockout mice: altered disposition of the dietary carcinogen PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) and its genotoxic metabolites. *Mol Pharmacol* 85: 520–530, 2014
37. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G: Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 323: 1718–1722, 2009
38. Brady KP, Dushkin H, Förnzler D, Koike T, Magner F, Her H, Gullans S, Segre GV, Green RM, Beier DR: A novel putative transporter maps to the osteosclerosis (oc) mutation and is not expressed in the oc mutant mouse. *Genomics* 56: 254–261, 1999
39. Vallon V, Rieg T, Ahn SY, Wu W, Eraly SA, Nigam SK: Overlapping in vitro and in vivo specificities of the organic anion transporters OAT1 and OAT3 for loop and thiazide diuretics. *Am J Physiol Renal Physiol* 294: F867–F873, 2008
40. Vanwert AL, Bailey RM, Sweet DH: Organic anion transporter 3 (Oat3/Slc22a8) knockout mice exhibit altered clearance and distribution of penicillin G. *Am J Physiol Renal Physiol* 293: F1332–F1341, 2007
41. Nagle MA, Truong DM, Dnyanmote AV, Ahn SY, Eraly SA, Wu W, Nigam SK: Analysis of three-dimensional systems for developing and mature kidneys clarifies the role of OAT1 and OAT3 in antiviral handling. *J Biol Chem* 286: 243–251, 2011
42. Truong DM, Kaler G, Khandelwal A, Swaan PW, Nigam SK: Multi-level analysis of organic anion transporters 1, 3, and 6 reveals major differences in structural determinants of antiviral discrimination. *J Biol Chem* 283: 8654–8663, 2008
43. Bekersky I, Popick AC: Disposition of bumetanide in the isolated perfused rat kidney: effects of probenecid and dose response. *Am J Cardiol* 57: 33A–37A, 1986
44. Tucker BJ, Blantz RC: Effect of furosemide administration on glomerular and tubular dynamics in the rat. *Kidney Int* 26: 112–121, 1984
45. Burnell JM, Kirby WM: Effectiveness of a new compound, benemid, in elevating serum penicillin concentrations. *J Clin Invest* 30: 697–700, 1951
46. Lesiński J, Dzierzanowska D, Szymczak M, Linda H, Wiśniewska C, Nawara A, Winogrodzka A: Treatment of gonorrhoea with procaine penicillin plus probenecid. *Br J Vener Dis* 49: 358–361, 1973
47. Robbins N, Koch SE, Tranter M, Rubinstein J: The history and future of probenecid. *Cardiovasc Toxicol* 12: 1–9, 2012
48. Aslamkhan AG, Han YH, Yang XP, Zalups RK, Pritchard JB: Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol* 63: 590–596, 2003
49. Di Giusto G, Anzai N, Ruiz ML, Endou H, Torres AM: Expression and function of Oat1 and Oat3 in rat kidney exposed to mercuric chloride. *Arch Toxicol* 83: 887–897, 2009
50. Engström K, Ameer S, Bernaudat L, Drasch G, Baeuml J, Skerfving S, Bose-O'Reilly S, Broberg K: Polymorphisms in genes encoding potential mercury transporters and urine mercury concentrations in populations exposed to mercury vapor from gold mining. *Environ Health Perspect* 121: 85–91, 2013
51. Zalups RK, Ahmad S: Homocysteine and the renal epithelial transport and toxicity of inorganic mercury: Role of basolateral transporter organic anion transporter 1. *J Am Soc Nephrol* 15: 2023–2031, 2004
52. Torres AM, Dnyanmote AV, Bush KT, Wu W, Nigam SK: Deletion of multispecific organic anion transporter Oat1/Slc22a6 protects against mercury-induced kidney injury. *J Biol Chem* 286: 26391–26395, 2011
53. Bakhiya N, Arlt VM, Bahn A, Burckhardt G, Phillips DH, Glatt H: Molecular evidence for an involvement of organic anion transporters (OATs) in aristolochic acid nephropathy. *Toxicology* 264: 74–79, 2009
54. Stefanović V, Polenaković M: Fifty years of research in Balkan endemic nephropathy: Where are we now? *Nephron Clin Pract* 112: c51–c56, 2009
55. Balachandran P, Wei F, Lin RC, Khan IA, Pasco DS: Structure activity relationships of aristolochic acid analogues: Toxicity in cultured renal epithelial cells. *Kidney Int* 67: 1797–1805, 2005
56. Nakagawa H, Hirata T, Terada T, Jutabha P, Miura D, Harada KH, Inoue K, Anzai N, Endou H, Inui K, Kanai Y, Koizumi A: Roles of organic anion transporters in the renal excretion of perfluorooctanoic acid. *Basic Clin Pharmacol Toxicol* 103: 1–8, 2008
57. Toyohara T, Akiyama Y, Suzuki T, Takeuchi Y, Mishima E, Tanemoto M, Momose A, Toki N, Sato H, Nakayama M, Hozawa A, Tsuji I, Ito S, Soga T, Abe T: Metabolomic profiling of uremic solutes in CKD patients. *Hypertens Res* 33: 944–952, 2010
58. Vanholder R, Abou-Deif O, Argiles A, Baurmeister U, Beige J, Brouckaert P, Brunet P, Cohen G, De Deyn PP, Drücke TB, Fliser D, Glorieux G, Herget-Rosenthal S, Hörl WH, Jankowski J, Jörres A, Massy ZA, Mischak H, Perna AF, Rodriguez-Portillo JM, Spasovski G, Stegmayr BG, Stenvinkel P, Thornalley PJ,

- Wanner C, Wiecek A: The role of EUTox in uremic toxin research. *Semin Dial* 22: 323–328, 2009
59. Vanholder R, Van Laecke S, Glorieux G: What is new in uremic toxicity? *Pediatr Nephrol* 23: 1211–1221, 2008
 60. Vitetta L, Linnane AW, Gobe GC: From the gastrointestinal tract (GIT) to the kidneys: Live bacterial cultures (probiotics) mediating reductions of uremic toxin levels via free radical signaling. *Toxins (Basel)* 5: 2042–2057, 2013
 61. Wikoff WR, Nagle MA, Kouznetsova VL, Tsigelny IF, Nigam SK: Untargeted metabolomics identifies enterobiome metabolites and putative uremic toxins as substrates of organic anion transporter 1 (Oat1). *J Proteome Res* 10: 2842–2851, 2011
 62. Masereeuw R, Mutsaers HA, Toyohara T, Abe T, Jhavar S, Sweet DH, Lowenstein J: The kidney and uremic toxin removal: Glomerulus or tubule? *Semin Nephrol* 34: 191–208, 2014
 63. Toyohara T, Suzuki T, Goto J, Ito S, Hishinuma T, Rubera I, Tauc M, Shiwaku HO, Takeuchi Y, Mishima E, Abe M, Tanemoto M, Masuda S, Kawano H, Maemura K, Nakayama M, Sato H, Mikkaichi T, Yamaguchi H, Fukui S, Fukumoto Y, Shimokawa H, Inui K, Terasaki T, Goto J, Ito S, Hishinuma T, Rubera I, Tauc M, Fujii-Kuriyama Y, Yabuuchi H, Moriyama Y, Soga T, Abe T: SLCO4C1 transporter eliminates uremic toxins and attenuates hypertension and renal inflammation. *J Am Soc Nephrol* 20: 2546–2555, 2009
 64. Reyes M, Benet LZ: Effects of uremic toxins on transport and metabolism of different biopharmaceutics drug disposition classification system xenobiotics. *J Pharm Sci* 100: 3831–3842, 2011
 65. Wu W, Dnyanmote AV, Nigam SK: Remote communication through solute carriers and ATP binding cassette drug transporter pathways: An update on the remote sensing and signaling hypothesis. *Mol Pharmacol* 79: 795–805, 2011
 66. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A* 106: 3698–3703, 2009
 67. Ahn SY, Jamshidi N, Mo ML, Wu W, Eraly SA, Dnyanmote A, Bush KT, Gallegos TF, Sweet DH, Palsson BO, Nigam SK: Linkage of organic anion transporter-1 to metabolic pathways through integrated “omics”-driven network and functional analysis. *J Biol Chem* 286: 31522–31531, 2011
 68. Eraly SA, Vallon V, Rieg T, Gangoiti JA, Wikoff WR, Siuzdak G, Barshop BA, Nigam SK: Multiple organic anion transporters contribute to net renal excretion of uric acid. *Physiol Genomics* 33: 180–192, 2008
 69. Ahn SY, Nigam SK: Toward a systems level understanding of organic anion and other multispecific drug transporters: a remote sensing and signaling hypothesis. *Mol Pharmacol* 76: 481–490, 2009
 70. Mori K, Ogawa Y, Ebihara K, Aoki T, Tamura N, Sugawara A, Kuwahara T, Ozaki S, Mukoyama M, Tashiro K, Tanaka I, Nakao K: Kidney-specific expression of a novel mouse organic cation transporter-like protein. *FEBS Lett* 417: 371–374, 1997
 71. Sakurai H: Urate transporters in the genomic era. *Curr Opin Nephrol Hypertens* 22: 545–550, 2013
 72. Mount DB: The kidney in hyperuricemia and gout. *Curr Opin Nephrol Hypertens* 22: 216–223, 2013
 73. Sautin YY, Johnson RJ: Uric acid: The oxidant-antioxidant paradox. *Nucleosides Nucleotides Nucleic Acids* 27: 608–619, 2008
 74. Eraly SA, Hamilton BA, Nigam SK: Organic anion and cation transporters occur in pairs of similar and similarly expressed genes. *Biochem Biophys Res Commun* 300: 333–342, 2003
 75. Gallegos TF, Martovetsky G, Kouznetsova V, Bush KT, Nigam SK: Organic anion and cation SLC22 “drug” transporter (Oat1, Oat3, and Oct1) regulation during development and maturation of the kidney proximal tubule. *PLoS One* 7: e40796, 2012
 76. Maeda A, Tsuruoka S, Kanai Y, Endou H, Saito K, Miyamoto E, Fujimura A: Evaluation of the interaction between nonsteroidal anti-inflammatory drugs and methotrexate using human organic anion transporter 3-transfected cells. *Eur J Pharmacol* 596: 166–172, 2008
 77. Eraly SA, Blantz RC, Bhatnagar V, Nigam SK: Novel aspects of renal organic anion transporters. *Curr Opin Nephrol Hypertens* 12: 551–558, 2003
 78. Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM: Inhibition of P-glycoprotein-mediated drug transport: A unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* 99: 552–557, 1999
 79. Nigam SK, Burton ME, Vasko MR: Quinidine-induced digoxin toxicity after discontinuing digoxin in a patient with renal failure. *Clin Pharm* 3: 662–664, 1984
 80. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ, Zhang L: International Transporter Consortium: Membrane transporters in drug development. *Nat Rev Drug Discov* 9: 215–236, 2010
 81. Kouznetsova VL, Tsigelny IF, Nagle MA, Nigam SK: Elucidation of common pharmacophores from analysis of targeted metabolites transported by the multispecific drug transporter-Organic anion transporter1 (Oat1). *Bioorg Med Chem* 19: 3320–3340, 2011
 82. Bhatnagar V, Xu G, Hamilton BA, Truong DM, Eraly SA, Wu W, Nigam SK: Analyses of 5′ regulatory region polymorphisms in human SLC22A6 (OAT1) and SLC22A8 (OAT3). *J Hum Genet* 51: 575–580, 2006
 83. Xu G, Bhatnagar V, Wen G, Hamilton BA, Eraly SA, Nigam SK: Analyses of coding region polymorphisms in apical and basolateral human organic anion transporter (OAT) genes [OAT1 (NKT), OAT2, OAT3, OAT4, URAT (RST)]. *Kidney Int* 68: 1491–1499, 2005
 84. Choudhuri S, Klaassen CD: Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. *Int J Toxicol* 25: 231–259, 2006
 85. Hesselson SE, Matsson P, Shima JE, Fukushima H, Yee SW, Kobayashi Y, Gow JM, Ha C, Ma B, Poon A, Johns SJ, Stryke D, Castro RA, Tahara H, Choi JH, Chen L, Picard N, Sjödin E, Roelofs MJ, Ferrin TE, Myers R, Kroetz DL, Kwok PY, Giacomini KM: Genetic variation in the proximal promoter of ABC and SLC superfamilies: Liver and kidney specific expression and promoter activity predict variation. *PLoS One* 4: e6942, 2009
 86. Han YF, Fan XH, Wang XJ, Sun K, Xue H, Li WJ, Wang YB, Chen JZ, Zhen YS, Zhang WL, Zhou X, Hui R: Association of intergenic polymorphism of organic anion transporter 1 and 3 genes with hypertension and blood pressure response to hydrochlorothiazide. *Am J Hypertens* 24: 340–346, 2011
 87. Yee SW, Nguyen AN, Brown C, Savic RM, Zhang Y, Castro RA, Cropp CD, Choi JH, Singh D, Tahara H, Stocker SL, Huang Y, Brett CM, Giacomini KM: Reduced renal clearance of cefotaxime in Asians with a low-frequency polymorphism of OAT3 (SLC22A8). *J Pharm Sci* 102: 3451–3457, 2013
 88. Fujita T, Brown C, Carlson EJ, Taylor T, de la Cruz M, Johns SJ, Stryke D, Kawamoto M, Fujita K, Castro R, Chen CW, Lin ET, Brett CM, Burchard EG, Ferrin TE, Huang CC, Leabman MK, Giacomini KM: Functional analysis of polymorphisms in the organic anion transporter, SLC22A6 (OAT1). *Pharmacogenet Genomics* 15: 201–209, 2005
 89. Lopez-Lopez E, Ballesteros J, Piñan MA, Sanchez de Toledo J, Garcia de Andoin N, Garcia-Miguel P, Navajas A, Garcia-Orad A: Polymorphisms in the methotrexate transport pathway: A new tool for MTX plasma level prediction in pediatric acute lymphoblastic leukemia. *Pharmacogenet Genomics* 23: 53–61, 2013
 90. Chen Y, Li S, Brown C, Cheatham S, Castro RA, Leabman MK, Urban TJ, Chen L, Yee SW, Choi JH, Huang Y, Brett CM, Burchard EG, Giacomini KM: Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. *Pharmacogenet Genomics* 19: 497–504, 2009
 91. Christensen MM, Pedersen RS, Stage TB, Brasch-Andersen C, Nielsen F, Damkier P, Beck-Nielsen H, Brøsen K: A gene-gene interaction between polymorphisms in the OCT2 and MATE1 genes influences the renal clearance of metformin. *Pharmacogenet Genomics* 23: 526–534, 2013
 92. Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, Sparreboom A: Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther* 86: 396–402, 2009

93. Schreuder MF, Bueters RR, Huigen MC, Russel FG, Masereeuw R, van den Heuvel LP: Effect of drugs on renal development. *Clin J Am Soc Nephrol* 6: 212–217, 2011
94. Pavlova A, Sakurai H, Leclercq B, Beier DR, Yu AS, Nigam SK: Developmentally regulated expression of organic ion transporters NKT (OAT1), OCT1, NLT (OAT2), and Roct. *Am J Physiol Renal Physiol* 278: F635–F643, 2000
95. Sweet DH, Eraly SA, Vaughn DA, Bush KT, Nigam SK: Organic anion and cation transporter expression and function during embryonic kidney development and in organ culture models. *Kidney Int* 69: 837–845, 2006
96. Sweeney DE, Vallon V, Rieg T, Wu W, Gallegos TF, Nigam SK: Functional maturation of drug transporters in the developing, neonatal, and postnatal kidney. *Mol Pharmacol* 80: 147–154, 2011
97. Hirsch GH, Hook JB: Maturation of renal organic acid transport: Substrate stimulation by penicillin and p-aminohippurate (PAH). *J Pharmacol Exp Ther* 171: 103–108, 1970
98. Hook JB, Hewitt WR: Development of mechanisms for drug excretion. *Am J Med* 62: 497–506, 1977
99. Joseph S, Nicolson TJ, Hammons G, Word B, Green-Knox B, Lyn-Cook B: Expression of drug transporters in human kidney: Impact of sex, age, and ethnicity. *Biol Sex Differ* 6: 4, 2015
100. McLean AJ, Le Couteur DG: Aging biology and geriatric clinical pharmacology. *Pharmacol Rev* 56: 163–184, 2004
101. Deguchi T, Ohtsuki S, Otagiri M, Takanaga H, Asaba H, Mori S, Terasaki T: Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. *Kidney Int* 61: 1760–1768, 2002
102. Takeda M, Babu E, Narikawa S, Endou H: Interaction of human organic anion transporters with various cephalosporin antibiotics. *Eur J Pharmacol* 438: 137–142, 2002
103. Goldstein RS, Smith PF, Tarloff JB, Contardi L, Rush GF, Hook JB: Biochemical mechanisms of cephaloridine nephrotoxicity. *Life Sci* 42: 1809–1816, 1988
104. Kohler JJ, Hosseini SH, Green E, Abuin A, Ludaway T, Russ R, Santoianni R, Lewis W: Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Lab Invest* 91: 852–858, 2011
105. Kone BC: The metabolic basis of solute transport. In: *Brenner and Rector's The Kidney*, 7th Ed., edited by Brenner BM, Philadelphia, Saunders, 2004, pp 231–260
106. Ruegg CE, Mandel LJ: Bulk isolation of renal PCT and PST. II. Differential responses to anoxia or hypoxia. *Am J Physiol* 259: F176–F185, 1990
107. Cropp CD, Komori T, Shima JE, Urban TJ, Yee SW, More SS, Giacomini KM: Organic anion transporter 2 (SLC22A7) is a facilitative transporter of cGMP. *Mol Pharmacol* 73: 1151–1158, 2008
108. Zhou F, Illsley NP, You G: Functional characterization of a human organic anion transporter hOAT4 in placental BeWo cells. *Eur J Pharm Sci* 27: 518–523, 2006
109. Kaler G, Truong DM, Sweeney DE, Logan DW, Nagle M, Wu W, Eraly SA, Nigam SK: Olfactory mucosa-expressed organic anion transporter, Oat6, manifests high affinity interactions with odorant organic anions. *Biochem Biophys Res Commun* 351: 872–876, 2006
110. Shiraya K, Hirata T, Hatano R, Nagamori S, Wiriyaermkul P, Jutabha P, Matsubara M, Muto S, Tanaka H, Asano S, Anzai N, Endou H, Yamada A, Sakurai H, Kanai Y: A novel transporter of SLC22 family specifically transports prostaglandins and co-localizes with 15-hydroxyprostaglandin dehydrogenase in renal proximal tubules. *J Biol Chem* 285: 22141–22151, 2010
111. Zhang Q, Hong M, Duan P, Pan Z, Ma J, You G: Organic anion transporter OAT1 undergoes constitutive and protein kinase C-regulated trafficking through a dynamin- and clathrin-dependent pathway. *J Biol Chem* 283: 32570–32579, 2008
112. Wu W, Bush KT, Liu HC, Zhu C, Abagyan R, Nigam SK: Shared ligands between organic anion transporters (Oat1 and Oat6) and odorant receptors. *Drug Metab Dispos* 43: 1–9, 2015
113. Zhu C, Nigam KB, Date RC, Springer SA, Bush KT, Saier MH Jr, Wu W, Nigam SK: Evolutionary analysis and classification of OATs, OCTs, OCTNs and other SLC22 transporters: Structure-function implications and analysis of sequence motifs. *PLoS One* 2015, in press

Received: March 7, 2014 Accepted: September 28, 2014

Published online ahead of print. Publication date available at www.cjasn.org.



Acid-Base Homeostasis

L. Lee Hamm,*[†] Nazih Nakhoul,*[†] and Kathleen S. Hering-Smith*[†]

Abstract

Acid-base homeostasis and pH regulation are critical for both normal physiology and cell metabolism and function. The importance of this regulation is evidenced by a variety of physiologic derangements that occur when plasma pH is either high or low. The kidneys have the predominant role in regulating the systemic bicarbonate concentration and hence, the metabolic component of acid-base balance. This function of the kidneys has two components: reabsorption of virtually all of the filtered HCO_3^- and production of new bicarbonate to replace that consumed by normal or pathologic acids. This production or generation of new HCO_3^- is done by net acid excretion. Under normal conditions, approximately one-third to one-half of net acid excretion by the kidneys is in the form of titratable acid. The other one-half to two-thirds is the excretion of ammonium. The capacity to excrete ammonium under conditions of acid loads is quantitatively much greater than the capacity to increase titratable acid. Multiple, often redundant pathways and processes exist to regulate these renal functions. Derangements in acid-base homeostasis, however, are common in clinical medicine and can often be related to the systems involved in acid-base transport in the kidneys.

Clin J Am Soc Nephrol 10: 2232–2242, 2015. doi: 10.2215/CJN.07400715

*Department of Medicine, Section of Nephrology, Tulane Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, Louisiana; and [†]Medicine Service, Southeast Louisiana Veterans Health Care System, New Orleans, Louisiana

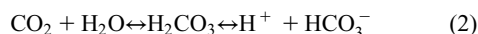
Acid-base homeostasis and pH regulation are critical for both normal physiology and cell metabolism and function. Normally, systemic acid-base balance is well regulated with arterial pH between 7.36 and 7.44; intracellular pH is usually approximately 7.2. For instance, chronic metabolic acidosis can be associated with decreased bone density, nephrolithiasis, muscle wasting, and progression of CKD (1–3). On a cellular level, many essential cellular processes, metabolic enzymes, and transmembrane transport processes are highly pH sensitive. Although this review will address systemic pH regulation and the role of the kidneys, individual cells also have a variety of mechanisms to regulate their intracellular pH (4). Overall concepts will be emphasized rather than specific pathways or processes, which are well covered elsewhere; references are selective.

Basic Concepts

Intracellular and extracellular buffers are the most immediate mechanism of defense against changes in systemic pH. Bone and proteins constitute a substantial proportion of these buffers. However, the most important buffer system is the $\text{HCO}_3^-/\text{CO}_2$ buffer system. The Henderson–Hasselbach equation (Equation 1) describes the relationship of pH, bicarbonate (HCO_3^-), and P_{CO_2} :

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03 \times \text{P}_{\text{CO}_2}} \quad (1)$$

where HCO_3^- is in milliequivalents per liter and P_{CO_2} is in millimeters of mercury. Equation 2 represents the reaction (water $[\text{H}_2\text{O}]$):



This buffer system is physiologically most important because of its quantitative capacity to buffer acid or alkali loads and because of the capacity for independent regulation of HCO_3^- and P_{CO_2} by the kidneys and lungs, respectively. In fact, this latter aspect of independent regulation is the most powerful aspect of this system. Although the lungs and kidneys can compensate for disorders of the other, normal homeostasis requires that both CO_2 and HCO_3^- be normal. Disorders of CO_2 are usually referred to as respiratory disorders, and disorders of HCO_3^- or fixed acids are referred to as metabolic disorders.

Arterial CO_2 is predominantly regulated by alveolar ventilation after production in peripheral tissues; CO_2 is often referred to as a gaseous acid, because its addition to aqueous solutions produces carbonic acid, which then releases H^+ and HCO_3^- (Equation 2 driven to the right). Plasma HCO_3^- is predominantly regulated by renal acid-base handling, and it will be discussed extensively below. The kidneys reabsorb, produce, and in some circumstances, excrete HCO_3^- . Plasma HCO_3^- is normally consumed daily by dietary acids and metabolic acids. As expressed by Equation 1, raising HCO_3^- or lowering P_{CO_2} will raise systemic pH, and lowering HCO_3^- or raising P_{CO_2} will lower pH.

In physiologic systems, the addition of an acid and the loss of alkali are essentially equivalent; for instance, as obvious in Equation 2, loss of HCO_3^- will pull the equation to the right, producing more H^+ . A frequent example of this is the loss of HCO_3^- with diarrhea or proximal renal tubular acidosis. Conversely, the physiologic addition of alkali and the loss of acid are

Correspondence:

Dr. L. Lee Hamm, Tulane University, 131 South Robertson, Suite 1500, 15th Floor, New Orleans, LA 70112. Email: Lhamm@tulane.edu

essentially equivalent. Therefore, the excretion of acid by the kidneys is equivalent to the production of base or HCO_3^- ; this point becomes important in considering below how the kidneys produce more HCO_3^- .

Typical high-animal protein Western diets and endogenous metabolism produce acid, typically on the order of 1 mEq/kg body wt per day or approximately 70 mEq/d for a 70-kg person. Phosphoric acid and sulfuric acid are significant products of this normal metabolism of dietary nutrients, such as proteins and phospholipids. To maintain acid-base homeostasis, these nonvolatile acids must be excreted by the kidney. Other nonvolatile acids, such as ketoacids and lactic acids, are produced in pathologic conditions. Nonvolatile acid loads (or loss of HCO_3^- , which is an equivalent process) in excess of the excretory capacity of the kidneys cause metabolic acidosis. Of note, vegetarian diets with high fruit and vegetable content are not acid producing and may produce a net alkali load (5); this may be an important consideration in the progression or treatment of CKD (6). Although the kidneys normally control plasma HCO_3^- , a few other factors have been considered. Endogenous acid production may be regulated, at least under certain circumstances (7); for instance, lactic acid and ketoacid production are decreased by a low pH. Also, hepatic production of HCO_3^- in the metabolism of proteins and amino acids is altered by systemic acid-base balance. Therefore, a role for hepatic contribution to the control of plasma HCO_3^- has been hypothesized (8).

Respiratory Control of P_{CO_2}

Alveolar ventilation normally eliminates approximately 15 mol CO_2 per day produced from normal cellular oxidative metabolism and maintains arterial P_{CO_2} around 40 mmHg. Normally, with increases (or decreases) in CO_2 production, alveolar ventilation increases (or decreases) to maintain P_{CO_2} and keep pH constant. Alveolar ventilation is controlled by chemoreceptor cells located in the medulla oblongata (and to a lesser extent, those in the carotid bodies), which are sensitive to pH and P_{CO_2} (4). Chemoreceptors respond to a decrease in cerebral interstitial pH by increasing ventilation and hence, lowering P_{CO_2} . Therefore, small increases in plasma CO_2 , which decrease pH, will result in stimulation of ventilation, which will normally rapidly return P_{CO_2} toward normal. Metabolic acidosis and the resulting acidemia will also result in an increase in ventilation (and lowering of P_{CO_2}) in response to decreases in cerebral interstitial pH. However, in contrast to the rapid response to changes in CO_2 , the response to nonvolatile acids or a change in plasma HCO_3^- is slower, because the central chemoreceptors are relatively insulated by the blood-brain barrier. Hence, acute changes in plasma HCO_3^- have a slower effect on cerebral interstitial pH and hence, stimulation of central chemoreceptors. This likely accounts for the 12- to 24-hour delay in the maximal ventilatory response to metabolic acid-base disturbances. The maximal ventilation response cannot usually reduce P_{CO_2} <10–12 mmHg (9–12). Decreases in ventilation may be limited because of a variety of clinical circumstances, such as lung disease, fluid overload, and central nervous system derangements. Also, decreases in P_{O_2} may limit the extent to which decreased ventilation can raise P_{CO_2} .

Renal Control of Plasma HCO_3^-

The kidneys have the predominant role of regulating the systemic HCO_3^- concentration and hence, the metabolic component of acid-base balance. This function of the kidneys has two components: reabsorption of virtually all of the filtered HCO_3^- and production of new HCO_3^- to replace that consumed by normal or pathologic acids. This production or generation of new HCO_3^- is done by net acid excretion. In other words, the kidneys make new HCO_3^- by excreting acid.

Because HCO_3^- is freely filtered at the glomerulus, approximately 4.5 mol HCO_3^- is normally filtered per day (HCO_3^- concentration of 25 mM/L $\times$ GFR of 0.120 L/min $\times$ 1440 min/d). Virtually all of this filtered HCO_3^- is reabsorbed, with the urine normally essentially free of HCO_3^- . Seventy to eighty percent of this filtered HCO_3^- is reabsorbed in the proximal tubule; the rest is reabsorbed along more distal segments of the nephron (Figure 1).

In addition to reabsorption of filtered HCO_3^- , the kidneys also produce additional HCO_3^- beyond that which has been filtered at the glomerulus. This process occurs by the excretion of acid into urine. (As indicated above, the excretion of acid is equivalent to the production of alkali.) The net acid excretion of the kidneys is quantitatively equivalent to the amount of HCO_3^- generation by the kidneys. Generation of new HCO_3^- by the kidneys is usually approximately 1 mEq/kg body wt per day (or about 70 mEq/d) and replaces that HCO_3^- that has been consumed by usual endogenous acid production (also about 70 mEq/d) as discussed above. During additional acid loads and in certain pathologic conditions, the kidneys increase the amount of acid excretion and the resulting new HCO_3^- generation. Net acid excretion by the kidneys occurs by two processes: the excretion of titratable acid and the excretion of ammonium (NH_4^+). Titratable acid refers to the excretion of protons with urinary buffers. The capacity of the nephron to excrete acid as free protons is limited as illustrated by the fact that the concentration of protons (H^+), even at a urine pH 4.5, is <0.1 mEq. However, the availability of urine

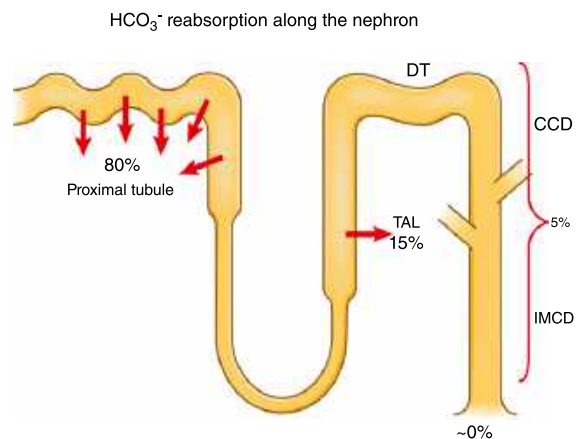


Figure 1. | Relative HCO_3^- transport along the nephron. Most of the filtered HCO_3^- is reabsorbed in the proximal tubule. Virtually no HCO_3^- remains in the final urine. CCD, cortical collecting duct; DT, distal convoluted tubule; IMCD, inner medullary collecting duct; TAL, thick ascending limb.

buffers (chiefly phosphate) results in the excretion of acid coupled to these urine buffers (13). Under normal conditions, approximately one-third to one-half of net acid excretion by the kidneys is in the form of titratable acid. The other one-half to two-thirds is the excretion of NH_4^+ . The mechanism whereby the excretion of NH_4^+ results in net acid excretion will be discussed below. The capacity to excrete NH_4^+ under conditions of acid loads is quantitatively much greater than the capacity to increase titratable acid (Figure 2) (14,15). Net acid excretion in the urine is, therefore, calculated as

$$\text{net acid excretion} = \text{titratable acid} + \text{ammonium} - \text{urinary } \text{HCO}_3^- \quad (3)$$

Loss of alkali in the urine in the form of HCO_3^- decreases the amount of net acid excretion or new HCO_3^- generation. Loss of organic anions, such as citrate, in the urine represents the loss of potential alkali or HCO_3^- . However, in humans, the loss of these organic anions is not usually quantitatively significant in whole-body acid-base balance (15).

Proximal Tubule HCO_3^- Reabsorption

The proximal tubule reabsorbs at least 70%–80% of the approximately 4500 mEq/d filtered HCO_3^- . The proximal tubule, therefore, has a high capacity for HCO_3^- reabsorption. The mechanisms of this reabsorption are illustrated in Figure 3. Most (probably >70%) of this HCO_3^- reabsorption occurs by proton secretion at the apical membrane by sodium-hydrogen exchanger NHE3 (16) (Figure 3). This protein exchanges one Na^+ ion for one H^+ ion, driven by the lumen to cell Na^+ gradient (approximately 140 mEq/L in the lumen and 15–20 mEq/L in the cell). The low intracellular Na^+ is maintained by the basolateral Na^+/K^+ -ATPase. An apical H^+ -ATPase and possibly, NHE8 under some circumstances account

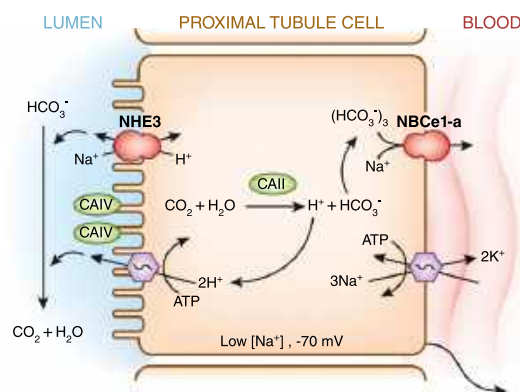


Figure 3. | Schematic representation of HCO_3^- reabsorption in the proximal tubule. Most H^+ secretion occurs through Na^+/H^+ exchange, although a component of an H^+ pump is also present. Carbonic anhydrase (CA), both intracellular and luminal, is important for HCO_3^- reabsorption. HCO_3^- exits the cell by NBCe1-A.

for the remaining portion of proximal tubule H^+ secretion and HCO_3^- reabsorption (17–19). This H^+ -ATPase shares most subunits with the distal tubule H^+ -ATPase described below.

In the lumen of the proximal tubule, the secreted H^+ reacts with luminal HCO_3^- to generate CO_2 and H_2O , which for the purposes here, can be considered to be freely permeable across the proximal tubule and reabsorbed. This reaction in the lumen (Equation 2 going from right to left) is relatively slow unless catalyzed, which occurs normally, by carbonic anhydrase (CA; in this case, membrane-bound CAIV tethered in the lumen). There are multiple isoforms of CA, but membrane-bound CAIV and cytosolic CAII are most important in renal acid-base transport (20,21). Mutations in CAII are known to cause a form of mixed proximal and distal renal tubular acidosis with osteopetrosis (22).

The source of the H^+ in the cells is the generation of H^+ and HCO_3^- from CO_2 and H_2O (Equation 2) catalyzed in the cell by intracellular CA, predominantly cytosolic CAII. The HCO_3^- generated within the proximal tubule cell by apical H^+ secretion exits across the basolateral membrane. Most of this HCO_3^- exit occurs by sodium HCO_3^- co-transport, NBCe1-A, or SLC4A4 (23). The stoichiometry and transported ionic species have been active areas of investigation, but studies suggest that 3 HCO_3^- Eq or 1 HCO_3^- Eq and 1 CO_3^{2-} Eq are transported with each Na^+ ; this electrogenic stoichiometry results in Na^+ and HCO_3^- being driven out of the cell by the cell-negative membrane voltage. Mutations in this (NBC-e1) protein cause proximal renal tubular acidosis (24). Chloride-bicarbonate exchange may also be present on the basolateral membrane of the proximal tubule but is not the main mechanism of HCO_3^- reabsorption.

The proximal tubule is a leaky epithelium on the basis of its particular tight junction proteins and therefore, unable to generate large transepithelial solute or electrical gradients; the minimal luminal pH and HCO_3^- obtained at the end of the proximal tubule are approximately pH 6.5–6.8 and 5 mM, respectively. The inability of the proximal tubule to lower pH further may also result from the

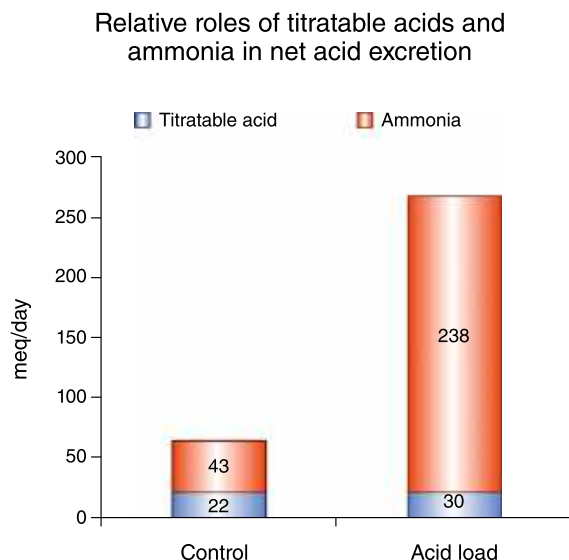


Figure 2. | Relative urinary titratable acid and ammonia in adults on a control or acid loading diet (with NH_4Cl). Reference 15.

dependence on the Na^+ gradient (140:15–20) driving the H^+ (pH) gradient. In the late proximal tubule, ongoing reabsorption of the low concentrations of luminal HCO_3^- may be countered and balanced by passive backleak of plasma or peritubular HCO_3^- into the lumen; however, continued Na^+/H^+ exchange activity will result in continued Na^+ reabsorption.

Regulation of HCO_3^- Reabsorption in the Proximal Tubule

A number of processes regulate proximal tubule HCO_3^- reabsorption both acutely and chronically. Many of these regulatory processes function to maintain acid-base homeostasis and are seemingly quite redundant; this is true in both the proximal and distal nephron segments. However, other processes overlap with volume or sodium regulatory processes, and the acid-base effects seem secondary and at times, dysfunctional for pH *per se*; for instance, during metabolic alkalosis induced by vomiting and volume depletion, various hormones are activated (catecholamines, angiotensin II, and aldosterone) that restore volume status but secondarily maintain high plasma HCO_3^- and alkalemia.

To maintain or restore acid-base balance, the proximal tubule increases HCO_3^- reabsorption during acidemia or acid loads and decreases HCO_3^- reabsorption during alkalemia or alkali loads (such that HCO_3^- might be excreted into the urine). Because this has been recently reviewed in this series (18), only a few comments will be made. The signals for these changes are often thought to be pH *per se*, either intracellular or extracellular. A variety of pH sensors have also been proposed (25), most notably including nonreceptor tyrosine kinase Pyk2, endothelin B receptor (activated by endogenous renal endothelin) (26,27), and CO_2 activation of ErbB1/2, ERK, and the apical angiotensin I receptor (28). More directly, decreased intracellular pH will increase the availability of protons for H^+ secretion and also, allosterically enhance the Na^+/H^+ exchanger (29). In addition to allosteric modulation of acid-base transporters, acute acidosis will cause insertion of additional transport proteins into the apical membrane, probably through the mechanisms immediately above; this has been best studied for NHE3. Chronic acidosis induces the production of additional transport proteins by increased transcription and production of mRNA; this is activated through a variety of signal transduction processes that are still being actively investigated. Both apical transport proteins (*e.g.*, NHE3) and basolateral transporters (*e.g.*, NBCe1-A) are often activated and increased in abundance in parallel, although the specifics may vary (30). Chronic potassium depletion also increases H^+ secretion, probably in response to changes in intracellular pH (31). In fact, hypokalemia has many effects on the kidney that parallel chronic acidosis, including effects on NH_4^+ excretion discussed below.

Extracellular fluid volume status is also an important determinant of proximal HCO_3^- reabsorption. Decreasing extracellular causes increased reabsorption of not only sodium but also, HCO_3^- , both in large part through increased Na-H exchange. Increases in volume status inhibit reabsorption of sodium and HCO_3^- , other factors being

unchanged. As mentioned above and discussed more below, clinically, this relates to the association of metabolic alkalosis with both volume contraction and edematous states (because these have decreased effective arterial volume). Volume expansion can be associated with decreased plasma HCO_3^- and metabolic acidosis, usually mild. An important additional component of the change in net HCO_3^- reabsorption with volume overload is an increased backleak of HCO_3^- into the tubule lumen. In contrast to the effects of volume, increasing delivery of HCO_3^- to the proximal tubule with constant extracellular volume results in a proportional increase in HCO_3^- reabsorption; this can be conceptualized as an increase in delivery to a reabsorptive system that is not saturated (*i.e.*, below the transport K_m , the Michaelis–Menten constant [the substrate concentration that gives half-maximal velocity of an enzymatic reaction and also, is used to model transport kinetics]).

A variety of hormones, some as above, affect proximal HCO_3^- reabsorption (32,33). Some of these hormones act on a short-term basis (response in minutes to hours), and others act over longer periods (days). On an acute basis, for instance, adrenergic agonists and angiotensin II stimulate HCO_3^- reabsorption (32). Parathyroid hormone acting through cAMP inhibits but hypercalcemia stimulates proximal HCO_3^- reabsorption. With these hormones and others, many studies have addressed the specific processes and signal transduction events (32). With chronic acid loads or acidosis, intrarenal endothelin-1 acting through the endothelin B receptor has been identified as a crucial element in the upregulation of Na^+/H^+ exchange (26,27). Glucocorticoids also are important in the development of proximal HCO_3^- transport and may be important in the chronic response to acidosis (34). In clinical circumstances, various regulatory factors may interact and be simultaneously operative. The net effect of these combined influences would vary depending on the exact circumstance. For instance, in metabolic alkalosis, volume contraction (and the associated hormonal changes and frequent fall in GFR) and high filtered loads of HCO_3^- increase proximal tubule HCO_3^- reabsorption, whereas increases in peritubular HCO_3^- and increased intracellular pH may be inhibiting HCO_3^- reabsorption—the net effect is maintenance of metabolic alkalosis until the volume status is corrected.

Despite the regulation mentioned above, changes in proximal HCO_3^- reabsorption may not be directly reflected in changes in whole-kidney net acid excretion or urinary HCO_3^- excretion, because additional HCO_3^- reabsorption and acid secretion occur in the distal nephron. Also, because virtually all of filtered HCO_3^- is normally reabsorbed by the kidneys, increases in HCO_3^- reabsorption *per se* cannot compensate for increased systemic acid loads. This compensation can only occur with increased acid excretion as titratable acids or NH_4^+ .

Distal Tubule Acidification

Several segments after the proximal tubule contribute substantially to acid-base homeostasis. First, the thick ascending limb (TAL) reabsorbs a significant amount of HCO_3^- , approximately 15% of the filtered load, predominantly through an apical Na^+/H^+ exchanger (35). TAL acid-base transport is regulated by a variety of factors

(e.g., Toll-like receptors, dietary salt, aldosterone, angiotensin II, *etc.* [36–38]), but the integrated role of the thick limb acid-base transport in various acid-base disorders has not been well clarified.

Beyond the TAL, the distal tubule compared with the proximal tubule has a limited capacity to secrete H^+ and thereby, reabsorb HCO_3^- . During inhibition of proximal tubule HCO_3^- reabsorption, increased distal delivery of HCO_3^- can overwhelm this limited reabsorptive capacity. However, the collecting tubule is able to generate a large transepithelial pH gradient (urine pH <5 with blood pH approximately 7.4). This large pH gradient is achievable because of the primary active pumps responsible for distal nephron H^+ secretion (discussed below) and because of the relative impermeability of the distal tubule to ions (*i.e.*, a tight epithelial membrane). The large pH gradient ensures the generation of titratable acid and the entrapment of NH_4^+ . The limitation in HCO_3^- reabsorption is likely, in part, caused by the absence of luminal CA discussed below, but there may be other factors as well, such as a limited number of H^+ pumps.

The distal tubule beyond the TAL consists of several distinct morphologic and functional segments, including the distal convoluted tubule, the connecting segment, and several distinct collecting duct segments, each with several cell types. Although several of these cell types can secrete H^+ , the CA-containing (and mitochondrial-rich) intercalated cells (ICs) are chiefly responsible for acid-base transport. There are at least three types of ICs: type A or α ICs, which secrete H^+ ; type B or β ICs, which secrete HCO_3^- ; and non-A, non-B ICs, with a range of function that

remains under investigation. These have been recently reviewed in this series (39) (Figure 4).

Conceptually, H^+ secretion in the type A ICs will result in HCO_3^- reabsorption in the presence of luminal HCO_3^- but will acidify the urine and generate new HCO_3^- in the absence of luminal HCO_3^- (*i.e.*, if virtually all of the filtered HCO_3^- has been reabsorbed upstream). The mechanism of this H^+ secretion involves primarily an apical H^+ -ATPase as illustrated in Figure 4. The apical H^+ -ATPase is an electrogenic active pump (vacuolar ATPase) able to secrete H^+ down to a urine pH of approximately 4.5. This is a multisubunit ATPase resembling that in intracellular organelles. The subunits are in two domains: a V_0 transmembrane domain and a V_1 cytosolic domain. Mutations in certain subunits are a known cause of distal renal tubular acidosis (40). Regulation of this pump is primarily by recycling between subapical vesicles and the plasma membrane involving the actin cytoskeleton and microtubules (41). Regulation may also be accomplished, in certain situations by assembly or disassembly of the two domains and phosphorylation of subunits. H^+ secretion also occurs through another set of pump(s): apical H^+/K^+ -ATPases. H^+/K^+ -ATPases in the collecting duct include both the gastric form of H^+/K^+ -ATPase and the colonic form of H^+/K^+ -ATPase (42). On the basis of inhibitor studies, there may also be a third type of H^+/K^+ -ATPase in the collecting duct (43,44). These transporters likely contribute to urine acidification under normal conditions but particularly during states of potassium depletion (42,44).

Conceptually, HCO_3^- reabsorption (or new HCO_3^- generation) as in the proximal tubule is a two-part process: secretion of H^+ into the lumen and HCO_3^- exit from the cell across the basolateral membrane. Therefore, to complete the process of HCO_3^- reabsorption or new HCO_3^- generation, HCO_3^- produced in the cell from CO_2 and H_2O exits across the basolateral membrane (Equation 2 as in the proximal tubule, with H^+ being secreted across the apical membrane). In other words, the HCO_3^- generated intracellularly by any of these pumps exits the basolateral membrane. This exit into the blood occurs through a chloride-bicarbonate exchanger, a truncated version of the anion exchanger 1 (AE1; band 3 protein), which is the $\text{Cl}^-/\text{HCO}_3^-$ exchanger in red blood cells that facilitates CO_2 transport (45). This is an electroneutral exchanger driven by the ion concentrations of chloride and HCO_3^- . Mutations in AE1 can also cause distal renal tubular acidosis (40,46). Two other transporters SLC26a7 (a $\text{Cl}^-/\text{HCO}_3^-$ exchanger) and KCC4 (a KCl cotransporter) are also in the basolateral membrane of some acid secreting cells in the collecting duct and likely contribute to urine acidification in certain conditions (47–49).

In the cortical collecting duct, in addition to HCO_3^- reabsorption by type A ICs, simultaneous HCO_3^- secretion occurs in separate cells, the type B ICs (39,50). This process involves an apical chloride-bicarbonate exchanger and a basolateral H^+ -ATPase (51). In animal studies, HCO_3^- secretion is stimulated by alkali loading and inhibited by low luminal chloride. The basolateral H^+ -ATPase is the same pump as in the apical membrane of type A IC, but the apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger has been identified as pendrin, a protein first identified as being abnormal in

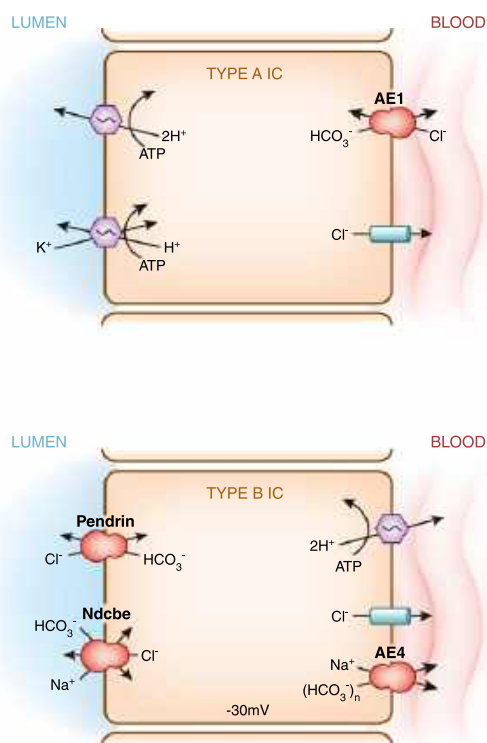


Figure 4. | Schematic of H^+ and HCO_3^- transport in the types A and B intercalated cells (ICs) in the collecting tubule (details are in the text). AE, anion exchanger.

some patients with hereditary deafness (39,52,53). The importance of HCO_3^- secretion in human pathophysiologic circumstances is not well characterized but may be important in the maintenance and/or repair of metabolic alkalosis.

The type B ICs, non-A, non-B ICs, and pendrin have now also been implicated in NaCl transport/balance and hypertension (53). This involves transport of Cl^- and HCO_3^- on pendrin and also, a sodium-driven chloride/ HCO_3^- exchanger (NDCBE or SLC4A8); Cl^- exits the cell on AE4 (54–57).

Cytosolic CA, CAII, is present in all ICs of the collecting tubule, and it facilitates provision of H^+ to be secreted into the lumen and HCO_3^- to be released into the basolateral aspect and blood. However, several segments of the distal nephron do not have luminal CA (*i.e.*, CAIV). The implications of this are that, as H^+ is secreted into the lumen, luminal pH may be below equilibrium pH, with pH, CO_2 , and HCO_3^- only coming to equilibrium later downstream in the final urine. Final urine P_{CO_2} may, therefore, rise above blood P_{CO_2} (as H^+ and HCO_3^- react and come to equilibrium with CO_2) and be an index of distal nephron H^+ secretion.

Regulation of Acid-Base Transporters in the Distal Nephron

Regulation of distal nephron acid-base transport is crucial, because this is the final site of control of urine composition. The distal tubule responds to systemic acidosis in a teleologically appropriate direction of increased H^+ secretion and new HCO_3^- generation. As in the proximal tubule, changes in intracellular pH and plasma P_{CO_2} affect proton secretion in the distal nephron. A significant portion of this increased H^+ secretion is through insertion of additional H^+ -ATPase by fusion of subapical vesicles (containing H^+ -ATPase) with the plasma membrane. These processes are mediated, in part, by a variety of signal transduction systems (cAMP, cGMP, and PLC/protein kinase C) (39,41). A role for soluble adenylyl cyclase, G protein-coupled receptor 4, and other mechanisms in sensing pH changes or acid-base loads has also been proposed in type A ICs and previously discussed in this series (39,41). Although the response to acidosis begins with these fairly rapid changes, chronic acidosis leads to a variety of changes in mRNA and protein levels of various transporters. Potassium depletion also increases H^+ secretion in the distal tubule, similar to changes in the proximal tubule.

The electrogenic nature of the apical H^+ -ATPase makes this process sensitive to transepithelial voltage. Therefore, an increased lumen-negative voltage (*e.g.*, more Na^+ reabsorption) will increase H^+ secretion. Increased Na^+ reabsorption, such as with increased mineralocorticoids, will, therefore, be accompanied by increased H^+ secretion. Interestingly, the cell voltages of ICs are strikingly different from most cells, including principal cells: first, although there is little data, the cell-negative voltages as shown in Figure 4 are much less (less negative), and second, the cells are energized by the H^+ pump rather than by Na-K-ATPase as in most cells (54,57,58). H^+ secretion, including in the medullary collecting tubule, is also directly stimulated by mineralocorticoids, not just by secondary electrical effects (59,60). Distal tubule acidification may be strongly

influenced by chloride concentration gradients between tubular lumen and peritubular blood; this may result both from voltage changes and by direct lumen to cell concentration gradients driving pendrin and other transporters.

In addition to aldosterone, other hormones and receptors, including angiotensin II and the calcium-sensing receptor, stimulate distal acidification (61). An important role for intrarenal endothelin has also been found (27,62,63).

Remodeling of IC morphology, number (relative numbers of A, B, and non-A, non-B ICs), and distribution of various transporters has also been characterized (64,65). The matrix protein hensen is a key mediator of this remodeling.

Ammonia Excretion

NH_4^+ excretion represents the other major mechanism whereby the kidneys excrete acid (66). Of the net acid excreted (or new HCO_3^- generated) by the kidneys, approximately one-half to two-thirds (approximately 40–50 mmol/d) is because of NH_4^+ excretion in the urine. Additionally, during acid loading or acidosis, NH_4^+ excretion can increase several fold, in contrast to a more limited increase in titratable acidity (15) (Figure 2).

Traditionally, NH_4^+ excretion was viewed as the excretion of protons in conjunction with a buffer (ammonia [NH_3]):



However, $\text{NH}_3/\text{NH}_4^+$ is not an effective physiologic buffer, because its pK_a is too high (approximately 9.2), and most of total ammonia (>98%) at physiologic pH is NH_4^+ on the basis of the pK_a . Furthermore, NH_4^+ , not NH_3 , is produced within the kidneys. However, total ammonia excretion in the urine does represent acid excretion (or the production of new HCO_3^-) when considered in conjunction with the metabolism of the deamidated carbon skeleton of glutamine. Glutamine is the predominant precursor of NH_3 in the kidney (Figure 5). Conceptually, the metabolism of the carbon skeleton of glutamine can result in the formation of one HCO_3^- formed for every NH_4^+ excreted. Other amino acids can also be used to some extent. The predominant enzymatic pathway for NH_3 formation is mitochondrial phosphate-dependent glutaminase in the proximal tubule, which produces one NH_4^+ and glutamate. Glutamate dehydrogenase can then convert glutamate into α -ketoglutarate and a second NH_4^+ . The α -ketoglutarate produced can be converted to glucose or completely metabolized to HCO_3^- . Other pathways for NH_3 production also exist but are thought to be less important. Other nephron segments other than the proximal tubule can produce NH_3 but not to the same extent, and the other segments do not have the same significant adaptive increases in ammoniogenesis with sustained acidosis as the proximal tubule. A variety of conditions affect the renal production of NH_3 , but the most important is that of acid-base status. Chronic metabolic acidosis can increase NH_3 production several fold in the kidneys. Potassium balance also alters NH_3 production, such that hyperkalemia suppresses NH_3 formation (and transport in both the proximal tubule and thick limb), and hypokalemia increases NH_3 production by the kidneys. Each of these changes has clinical correlates in overall acid-base balance. A variety

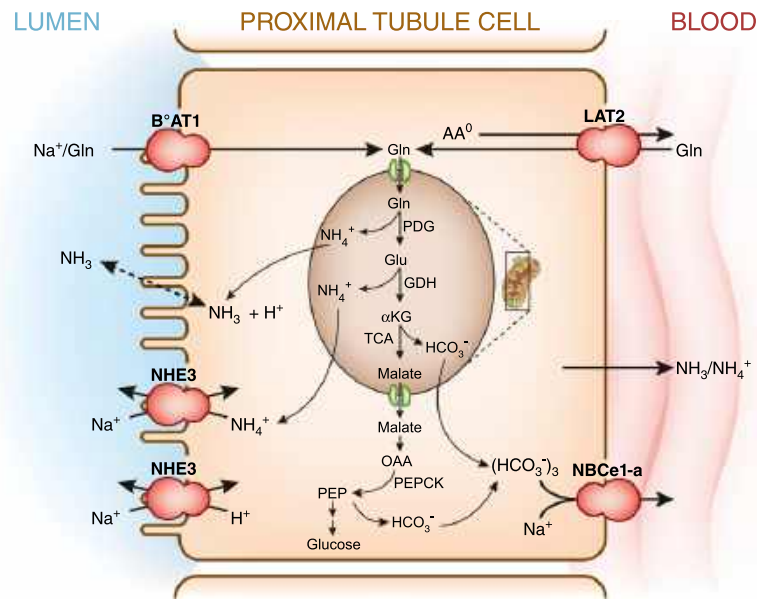


Figure 5. | Schematic of ammonia (NH_3) production in the proximal tubule. A proximal tubule cell is enlarged to show that glutamine (Gln) is metabolized to ammonium (NH_4^+) by phosphate-dependent glutaminase (PDG); through a series of steps, the carbon skeleton of glutamine can be metabolized to HCO_3^- . NH_3 can enter the proximal tubule lumen by either NH_3 diffusion or NH_4^+ movement on the Na^+ - H^+ exchanger. AA^0 , neutral amino acids; B*AT1, B-type neutral amino acids transporter; GDH, glutamate dehydrogenase; αKG , alpha keto-glutarate; LAT2, L-type amino acids transporter-2; OAA, oxalo-acetate; PEP, phospho enol-pyruvate; PEPCCK, phospho enol-pyruvate carboxy kinase; TCA, TCA cycle.

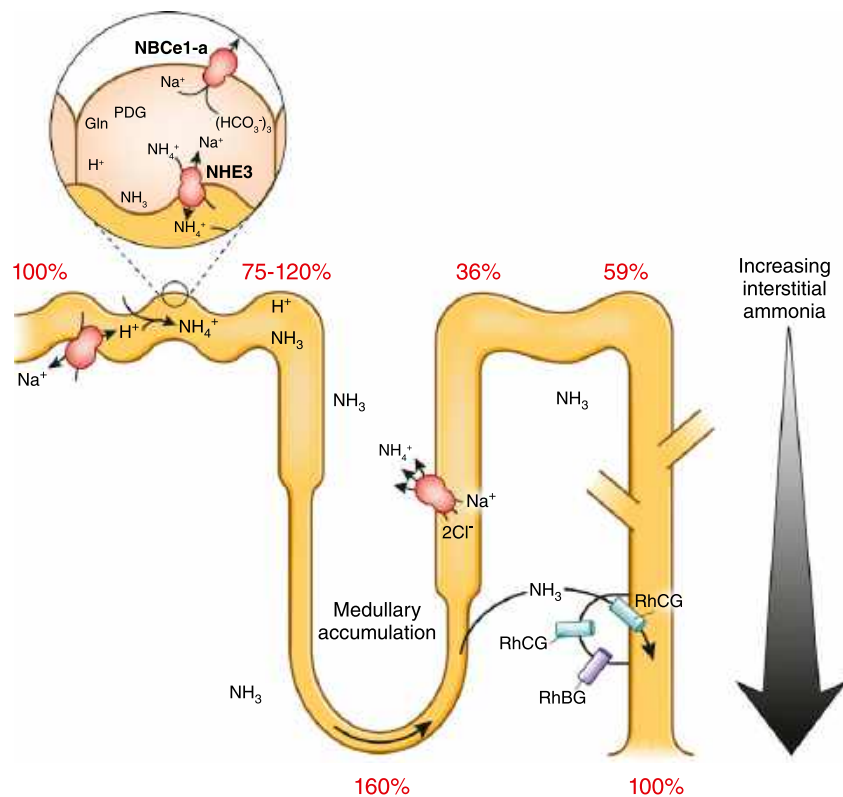


Figure 6. | Ammonium (NH_4^+) transport along the nephron. In the thick ascending limb, NH_4^+ is reabsorbed by the $\text{Na}^+\text{K}^+\text{2Cl}^-$ transporter. In the collecting duct, Rh proteins mediate ammonia (NH_3)/ NH_4^+ transport. The numbers depict the percentages of urinary total NH_3 at each site. Total NH_3 is concentrated in the medulla (details are in the text). Gln, glutamine; PDG, phosphate-dependent glutaminase.

of hormones (angiotensin II, prostaglandins, *etc.*) have also been shown to alter NH_3 production. The enzymatic and transporter changes that facilitate enhanced ammoniogenesis during acidosis have recently been reviewed in this series (18).

The transport of the produced NH_4^+ along the nephron and into the urine is a complex process that is still a topic of intense study (Figure 6). Most of the NH_4^+ produced is excreted into the urine rather than added to the venous blood; this is particularly true with acidosis—the proportions into urine and blood are almost even in normal conditions (13,67). Therefore, during acidosis, both total NH_3 production and secretion into urine are increased. The overall picture of $\text{NH}_3/\text{NH}_4^+$ transport is summarized as follows. In the proximal tubular, NH_4^+ is produced from glutamine as discussed above and then, secreted into the lumen. This secretion occurs by both $\text{Na}^+/\text{NH}_4^+$ exchange on the Na^+/H^+ exchanger and NH_3 diffusion across the apical membrane (68–70). Interestingly, the secretion of NH_4^+ across the apical membrane is augmented by angiotensin II (71). Whether the diffusion of NH_3 across the apical membrane of the proximal tubule is lipid-phase diffusion as long thought or facilitated by certain gas channels has not been determined. In the loop of Henle, NH_4^+ ion is reabsorbed into the interstitium, where it accumulates in the medulla. In the TAL, NH_4^+ is transported across the apical membrane predominantly on the $\text{Na}^+-\text{K}^+-2\text{Cl}$ cotransporter, with perhaps some entry on K^+ channels (72). NH_4^+ has a similar hydrated size as K^+ and therefore, can often be transported through many K^+ pathways (73). Remarkably, the apical membrane of the TAL is virtually impermeable to NH_3 in contrast to most cell membranes (74). NH_3 probably leaves the TAL cell across the basolateral membrane by NH_3 diffusion and NH_4^+ movement by NHE4 (72). One fraction of this medullary NH_4^+ is secreted back into the late proximal tubule as NH_3 diffusion coupled to H^+ secretion, and therefore, it is partially recycled. Another fraction of medullary NH_4^+ enters the lumen of the cortical and medullary collecting ducts and as such, bypasses the superficial/cortical distal segment of the nephron. A small fraction of interstitial NH_4^+ is shunted to the systemic circulation for eventual detoxification by the liver.

In the cortical and medullary collecting ducts, interstitial $\text{NH}_3/\text{NH}_4^+$ is secreted into the lumen by several mechanisms, most identified only in recent years. The classic mechanism involves diffusion of medullary NH_3 across the basolateral and apical membranes into the lumen, where secreted H^+ titrates NH_3 to NH_4^+ . Another fraction of transcellular NH_4^+ secretion involves the $\text{Na}^+-\text{K}^+-\text{ATPase}$ that carries NH_4^+ into the cell by substituting NH_4^+ for K^+ . NH_4^+ can also be transported by $\text{H}^+-\text{K}^+-\text{ATPases}$ in the collecting duct. Another critical mechanism of $\text{NH}_3/\text{NH}_4^+$ transport is Rh proteins, specifically RhBG and RhCG. These were only identified as $\text{NH}_3/\text{NH}_4^+$ transporters in recent years (75,76). These proteins function as $\text{NH}_3/\text{NH}_4^+$ transporters, clearly facilitating diffusion of NH_3 but also, probably including (at least for RhBG) electrogenic transport of NH_4^+ (77,78). RhBG is present in the basolateral membrane of type A ICs and can transport NH_4^+ and NH_3 into the cell, whereas RhCG, present at the apical membrane of several cell types (and in some

circumstances, basolateral membrane), transports NH_3 into the lumen. Luminal NH_3 is then titrated by secreted H^+ to NH_4^+ as described above.

Acid and Alkali Loads

With this background, what are the pathologic challenges to normal acid-base homeostasis? Table 1 provides a basic conceptual classification of pathophysiologic types of acid and alkali loads. This differs from the usual classifications of acid-base disorders *per se*, which focus only on clinical diagnostic algorithms or dive deeper into mechanisms. Acid loads can be in the form of either CO_2 or non-volatile acids. Both types of acid loads occur normally on a daily basis and must be excreted. Primary ventilatory disorders resulting in increased arterial P_{CO_2} are referred to by the term respiratory acidosis. Nonvolatile acids, such as phosphoric acid and sulfuric acid, are also normal by-products of metabolism of dietary nutrients, proteins, and phospholipids. Nonvolatile acid loads in excess of the excretory capacity of the kidneys produce conditions termed metabolic acidosis. In other words, with normal acid loads or some increased acid loads that develop slowly, the kidneys can adapt, increasing acid excretion by both titratable acid and NH_4^+ excretion, and maintain normal systemic acid-base homeostasis and pH. With larger acid loads (or base losses; for example, through severe diarrhea), the kidneys cannot keep up (*i.e.*, cannot excrete sufficient acid into the urine), and metabolic acidosis ensues. Metabolic acidosis is reflected predominantly by lowering of plasma or systemic HCO_3^- . Also, the loss of alkali from the body is equivalent to the addition of acid, and therefore, it represents metabolic acidosis; such loss of alkali might occur from excess stool HCO_3^- losses or loss of HCO_3^- in the urine.

Alkali loads, in contrast to acid loads, are not the result of normal physiology in persons on most diets, which provide net dietary acid. Alkali loads can be either respiratory or metabolic. Primary increases in ventilation and lowering of P_{CO_2} are referred to as respiratory alkalosis. Metabolic alkali loads can result from excess excretion of urinary acid, loss of other acids (such as gastric acid), or administration of exogenous alkali, and they can result in metabolic alkalosis. Metabolic alkalosis is reflected primarily by an increased plasma or systemic HCO_3^- .

Usually, however, as briefly discussed below, such metabolic alkali loads can be quickly excreted, so that the focus in understanding metabolic alkalosis is more on the factors that maintain the high plasma HCO_3^- and not the original generation by alkali load (79). Clinically, the factors that often maintain high plasma HCO_3^- include extracellular volume and chloride depletion, which may decrease GFR, increase proximal tubule HCO_3^- reabsorption, and increase angiotensin and mineralocorticoids, potent stimuli for H^+ secretion in the proximal and distal tubules, respectively, as discussed above. The intermediaries in volume depletion include increased sympathetic activity and increased catecholamines, angiotensin II, and aldosterone, all of which increase H^+ secretion, HCO_3^- reabsorption, and/or NH_4^+ excretion as discussed above. The classic edematous disorders of congestive heart failure and cirrhosis and some nephrotic syndrome also have effective extracellular volume depletion because of low

Table 1. Acid and alkali loads**Acid loads**CO₂: Respiratory acidosis

Nonvolatile acids: Metabolic acidosis

*Exogenous acids (e.g., salicylate, methanol, and ethylene glycol)**Pathologic endogenous acids (e.g., ketoacids and lactic acid)**Decreased renal acid excretion (e.g., renal failure and distal renal tubular acidosis)**Loss of alkali, equivalent to acid load*

Gastrointestinal losses (e.g., diarrhea)

Urine losses (e.g., proximal renal tubular acidosis)

Alkali loadsExcess CO₂ exhalation: Respiratory alkalosis

Nonvolatile alkali: Metabolic alkalosis

*Exogenous alkali (e.g., NaHCO₃ administration)**Loss of acid, equivalent to alkali load*

Gastrointestinal losses (e.g., gastric fluid)

Excess urine H⁺ losses and renal production of new HCO₃⁻ (most classic causes of metabolic alkalosis, including chloride depletion metabolic alkalosis and mineralocorticoid excess)

cardiac output or arterial underfilling (80); these conditions, thus, have sodium and H₂O retention but also, can have increased HCO₃⁻ reabsorption, H⁺ secretion, and/or NH₄⁺ excretion caused by the factors discussed above. Therefore, both volume depletion (e.g., vomiting and diuretics) and edematous disorders (primarily heart failure and cirrhosis) can and often are associated with metabolic alkalosis. Chloride depletion alone through a variety of mechanisms may also maintain metabolic alkalosis. Excess mineralocorticoids from any condition can also stimulate H⁺ secretion and maintain metabolic alkalosis. Potassium depletion contributes to the maintenance of metabolic alkalosis by stimulating continued H⁺ secretion.

Compensation for Acid-Base Disorders

The mechanisms of physiologic responses to acid or base loads can be expected on the basis of the understanding of the mechanisms of usual physiology described above. The predicted extent of clinical response, however, is on the basis of empirical observations and not just mechanisms. On the basis of the chemistry, acute changes in P_{CO2} alter plasma HCO₃⁻ slightly, because nonbicarbonate body buffers are titrated. With chronic changes in P_{CO2} (respiratory acid-base disorders), the kidneys respond to the change in pH by altering plasma HCO₃⁻ in a direction to lessen the change in systemic pH through the mechanisms described above. These changes within the kidney take several days for completion and in general, do not return systemic pH completely back to normal. With chronic hypocapnia and the resultant increase in systemic pH, decreases in reabsorption of HCO₃⁻ in the proximal tubule and distal tubule H⁺ secretion result in a fall in plasma HCO₃⁻; these changes can begin within a very few hours (81). With chronic hypercapnia, increased proximal and distal H⁺ secretion results in increased HCO₃⁻ reabsorption and increased production of new HCO₃⁻, resulting in a higher level of plasma HCO₃⁻; most of the cell and molecular mechanisms parallel the response to metabolic acid loads, where these have been studied (82). Increased NH₄⁺ excretion is expected as well with chronic respiratory acidosis (83). With the ubiquitous changes that occur in response to acidosis or acid loads, many investigators have looked for acid

sensors; a variety of proteins seem to serve some of this function, but no single sensor can be proposed (25).

Increased metabolic acid production or ingestion will initially result in increased new HCO₃⁻ generation, such that plasma HCO₃⁻ does not change. With larger acid loads, the capacity of the kidneys to generate new HCO₃⁻ is overwhelmed, and plasma HCO₃⁻ decreases. Metabolic acid loads or metabolic acidosis results in increased fractional proximal tubule HCO₃⁻ reabsorption, increases in distal H⁺ secretion (resulting in lower urine pH), and increased NH₄⁺ excretion. (However, the lower plasma HCO₃⁻ during metabolic acidosis decreases filtered HCO₃⁻ and hence, decreases absolute proximal HCO₃⁻ reabsorption.) Increased H⁺ excretion may result not only from adaptive changes in response to systemic pH *per se* but also, secondary to increases in systemic glucocorticoids and mineralocorticoids with chronic acidosis and potassium depletion. The adaptive changes within the kidney include various factors discussed in the sections above, including endogenous endothelin. The increased H⁺ secretion can result in increased titratable acid excretion up to 2- to 3-fold in certain situations. Urinary NH₄⁺ excretion (a result of both increased production and increased secretion of produced NH₄⁺) can increase several fold (Figure 2). The maximum renal compensation for acid loading requires 3–5 days. Metabolic acid loads sufficient to lower plasma pH result in increased ventilation, resulting in a decrease in P_{CO2} sufficient to raise the systemic pH toward but not to normal. The full respiratory compensation requires 12–24 hours. Paradoxically, the compensatory hypocapnia during metabolic acidosis may actually decrease somewhat the renal response to metabolic acidosis (84).

Metabolic alkali loads can normally be quickly eliminated in the urine, because the filtration rate of HCO₃⁻ is high, and a small reduction in proximal tubule HCO₃⁻ reabsorption can result in spillage of HCO₃⁻ in the urine and prompt correction of the metabolic alkalosis. Frequently, however, in states of metabolic alkalosis, other factors prevent the excretion of HCO₃⁻ and maintain the metabolic alkalosis as described above. Metabolic alkalosis also results in some hypoventilation and increase in P_{CO2} to compensate for the alkalemia.

Summary

Acid-base homeostasis is critical for normal physiology and health. Hence, multiple, often redundant pathways and processes exist to control systemic pH. Derangements in acid-base homeostasis, however, are common in clinical medicine and can often be related to the systems involved in acid-base transport in the kidneys. These have been studied for decades, but a variety of new pathways, such as pendrin and Rh proteins, have illustrated that our understanding is still far from complete.

Disclosures

None.

References

- Banerjee T, Crews DC, Wesson DE, Tilea A, Saran R, Rios Burrows N, Williams DE, Powe NR; Centers for Disease Control and Prevention Chronic Kidney Disease Surveillance Team: Dietary acid load and chronic kidney disease among adults in the United States. *BMC Nephrol* 15: 137, 2014
- Moe OW, Huang CL: Hypercalciuria from acid load: Renal mechanisms. *J Nephrol* 19 [Suppl 9]: S53–S61, 2006
- Wang XH, Mitch WE: Mechanisms of muscle wasting in chronic kidney disease. *Nat Rev Nephrol* 10: 504–516, 2014
- Ruffin VA, Salameh AI, Boron WF, Parker MD: Intracellular pH regulation by acid-base transporters in mammalian neurons. *Front Physiol* 5: 43, 2014
- Remer T, Manz F: Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc* 95: 791–797, 1995
- Goraya N, Wesson DE: Dietary interventions to improve outcomes in chronic kidney disease. *Curr Opin Nephrol Hypertens* 24: 505–510, 2015
- Hood VL, Tannen RL: Protection of acid-base balance by pH regulation of acid production. *N Engl J Med* 339: 819–826, 1998
- Häussinger D: Liver regulation of acid-base balance. *Miner Electrolyte Metab* 23: 249–252, 1997
- Adrogué HJ, Madias NE: Tools for clinical assessment. In: *Acid-Base Disorders and Their Treatment*, edited by Gennari FJ, Adrogué HJ, Galla JH, Madias NE, Boca Raton, FL, Taylor and Francis Group, 2005, pp 801–816
- Adrogué HJ, Madias NE: Secondary responses to altered acid-base status: The rules of engagement. *J Am Soc Nephrol* 21: 920–923, 2010
- Albert MS, Dell RB, Winters RW: Quantitative displacement of acid-base equilibrium in metabolic acidosis. *Ann Intern Med* 66: 312–322, 1967
- Javaheri S: Determinants of carbon dioxide tension. In: *Acid-Base Disorders and Their Treatment*, edited by Gennari FJ, Adrogué HJ, Galla JH, Madias NE, Boca Raton, FL, Taylor and Francis Group, 2005, pp 47–77
- Hamm LL, Simon EE: Roles and mechanisms of urinary buffer excretion. *Am J Physiol* 253: F595–F605, 1987
- Elkinton JR, Huth EJ, Webster GD Jr., McCance RA: The renal excretion of hydrogen ion in renal tubular acidosis. I. Quantitative assessment of the response to ammonium chloride as an acid load. *Am J Med* 29: 554–575, 1960
- Lemann Jr., Bushinsky DA, Hamm LL: Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol* 285: F811–F832, 2003
- Brant SR, Yun CH, Donowitz M, Tse CM: Cloning, tissue distribution, and functional analysis of the human Na⁺/H⁺ exchanger isoform, NHE3. *Am J Physiol* 269: C198–C206, 1995
- Baum M, Twombly K, Gattineni J, Joseph C, Wang L, Zhang Q, Dwarakanath V, Moe OW: Proximal tubule Na⁺/H⁺ exchanger activity in adult NHE8^{-/-}, NHE3^{-/-}, and NHE3^{-/-}/NHE8^{-/-} mice. *Am J Physiol Renal Physiol* 303: F1495–F1502, 2012
- Curthoys NP, Moe OW: Proximal tubule function and response to acidosis. *Clin J Am Soc Nephrol* 9: 1627–1638, 2014
- Li HC, Du Z, Barone S, Rubera I, McDonough AA, Tauc M, Zahedi K, Wang T, Soleimani M: Proximal tubule specific knockout of the Na⁺/H⁺ exchanger NHE3: Effects on bicarbonate absorption and ammonium excretion. *J Mol Med (Berl)* 91: 951–963, 2013
- Purkerson JM, Schwartz GJ: The role of carbonic anhydrases in renal physiology. *Kidney Int* 71: 103–115, 2007
- Schwartz GJ: Physiology and molecular biology of renal carbonic anhydrase. *J Nephrol* 15[Suppl 5]: S61–S74, 2002
- Alper SL: Genetic diseases of acid-base transporters. *Annu Rev Physiol* 64: 899–923, 2002
- Parker MD, Boron WF: The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. *Physiol Rev* 93: 803–959, 2013
- Igarashi T, Inatomi J, Sekine T, Cha SH, Kanai Y, Kunimi M, Tsukamoto K, Satoh H, Shimadzu M, Tozawa F, Mori T, Shiobara M, Seki G, Endou H: Mutations in SLC4A4 cause permanent isolated proximal renal tubular acidosis with ocular abnormalities. *Nat Genet* 23: 264–266, 1999
- Brown D, Wagner CA: Molecular mechanisms of acid-base sensing by the kidney. *J Am Soc Nephrol* 23: 774–780, 2012
- Laghmani K, Preisig PA, Alpern RJ: The role of endothelin in proximal tubule proton secretion and the adaptation to a chronic metabolic acidosis. *J Nephrol* 15[Suppl 5]: S75–S87, 2002
- Wesson DE: Endothelins and kidney acidification. *Contrib Nephrol* 172: 84–93, 2011
- Skelton LA, Boron WF: Effect of acute acid-base disturbances on the phosphorylation of phospholipase C-γ1 and Erk1/2 in the renal proximal tubule. *Physiol Rep* 3: e12280, 2015
- Aronson PS, Nee J, Suhm MA: Modifier role of internal H⁺ in activating the Na⁺-H⁺ exchanger in renal microvillus membrane vesicles. *Nature* 299: 161–163, 1982
- Kwon TH, Fulton C, Wang W, Kurtz I, Frøkjaer J, Aalkjaer C, Nielsen S: Chronic metabolic acidosis upregulates rat kidney Na-HCO cotransporters NBCn1 and NBC3 but not NBC1. *Am J Physiol Renal Physiol* 282: F341–F351, 2002
- Adam WR, Koretsky AP, Weiner MW: ³¹P-NMR in vivo measurement of renal intracellular pH: Effects of acidosis and K⁺ depletion in rats. *Am J Physiol* 251: F904–F910, 1986
- Hamm LL, Nakhoul NL: Renal acidification mechanisms. In: *The Kidney*, edited by Brenner B, Philadelphia, W.B. Saunders, 2008, pp 248–279
- Weiner ID, Verlander JW: Renal acidification mechanisms. In: *Brenner and Rector's The Kidney*, 9th Ed., edited by Brenner B, Philadelphia, W.B. Saunders, 2011, pp 293–325
- Bobulescu IA, Dwarakanath V, Zou L, Zhang J, Baum M, Moe OW: Glucocorticoids acutely increase cell surface Na⁺/H⁺ exchanger-3 (NHE3) by activation of NHE3 exocytosis. *Am J Physiol Renal Physiol* 289: F685–F691, 2005
- Capasso G, Unwin R, Rizzo M, Pica A, Giebisch G: Bicarbonate transport along the loop of Henle: Molecular mechanisms and regulation. *J Nephrol* 15[Suppl 5]: S88–S96, 2002
- Good DW, George T, Watts BA 3rd: High sodium intake increases HCO₃⁻ absorption in medullary thick ascending limb through adaptations in basolateral and apical Na⁺/H⁺ exchangers. *Am J Physiol Renal Physiol* 301: F334–F343, 2011
- Watts BA 3rd, George T, Good DW: Aldosterone inhibits apical NHE3 and HCO₃⁻ absorption via a nongenomic ERK-dependent pathway in medullary thick ascending limb. *Am J Physiol Renal Physiol* 291: F1005–F1013, 2006
- Watts BA 3rd, George T, Good DW: Lumen LPS inhibits HCO₃⁻ absorption in the medullary thick ascending limb through TLR4-PI3K-Akt-mTOR-dependent inhibition of basolateral Na⁺/H⁺ exchange. *Am J Physiol Renal Physiol* 305: F451–F462, 2013
- Roy A, Al-bataineh MM, Pastor-Soler NM: Collecting duct intercalated cell function and regulation. *Clin J Am Soc Nephrol* 10: 305–324, 2015
- Battle D, Haque SK: Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant* 27: 3691–3704, 2012
- Breton S, Brown D: Regulation of luminal acidification by the V-ATPase. *Physiology (Bethesda)* 28: 318–329, 2013
- Gumz ML, Lynch JJ, Greenlee MM, Cain BD, Wingo CS: The renal H⁺-K⁺-ATPases: Physiology, regulation, and structure. *Am J Physiol Renal Physiol* 298: F12–F21, 2010
- Buffin-Meyer B, Younes-Ibrahim M, Barlet-Bas C, Cheval L, Marsy S, Doucet A: K depletion modifies the properties of Sch-28080-sensitive K-ATPase in rat collecting duct. *Am J Physiol* 272: F124–F131, 1997
- Greenlee MM, Lynch JJ, Gumz ML, Cain BD, Wingo CS: The renal H,K-ATPases. *Curr Opin Nephrol Hypertens* 19: 478–482, 2010

45. Alper SL: Molecular physiology of SLC4 anion exchangers. *Exp Physiol* 91: 153–161, 2006
46. Alper SL: Familial renal tubular acidosis. *J Nephrol* 23[Suppl 16]: S57–S76, 2010
47. Alper SL, Sharma AK: The SLC26 gene family of anion transporters and channels. *Mol Aspects Med* 34: 494–515, 2013
48. Melo Z, Cruz-Rangel S, Bautista R, Vaquez N, Castaneda-Bueno M, Mount DB, Pasantes-Morales H, Mercado A, Gamga G: Molecular evidence for a role for K(+)-Cl(-) cotransporters in the kidney. *Am J Physiol Renal Physiol* 305: F1402–F1411, 2013
49. Soleimani M: SLC26 Cl⁻/HCO₃⁻ exchangers in the kidney: Roles in health and disease. *Kidney Int* 84: 657–666, 2013
50. Hamm LL, Hering-Smith KS, Vehaskari VM: Control of bicarbonate transport in collecting tubules from normal and remnant kidneys. *Am J Physiol* 256: F680–F687, 1989
51. Weiner ID, Hamm LL: Regulation of intracellular pH in the rabbit cortical collecting tubule. *J Clin Invest* 85: 274–281, 1990
52. Royaux IE, Wall SM, Karniski LP, Everett LA, Suzuki K, Knepper MA, Green ED: Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc Natl Acad Sci U S A* 98: 4221–4226, 2001
53. Wall SM, Lazo-Fernandez Y: The role of pendrin in renal physiology. *Annu Rev Physiol* 77: 363–378, 2015
54. Chambrey R, Kurth I, Peti-Peterdi J, Houillier P, Purkerson JM, Leviel F, Hentschke M, Zdebek AA, Schwartz GJ, Hübner CA, Eladari D: Renal intercalated cells are rather energized by a proton than a sodium pump. *Proc Natl Acad Sci U S A* 110: 7928–7933, 2013
55. Eladari D, Chambrey R, Picard N, Hadchouel J: Electroneutral absorption of NaCl by the aldosterone-sensitive distal nephron: Implication for normal electrolytes homeostasis and blood pressure regulation. *Cell Mol Life Sci* 71: 2879–2895, 2014
56. Leviel F, Hübner CA, Houillier P, Morla L, El Moghrabi S, Brideau G, Hassan H, Parker MD, Kurth I, Kougioumtzes A, Sinning A, Pech V, Riemondy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, Eladari D: The Na⁺-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electro-neutral Na⁺ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest* 120: 1627–1635, 2010
57. Koeppen BM: Electrophysiological identification of principal and intercalated cells in the rabbit outer medullary collecting duct. *Pflügers Arch* 409: 138–141, 1987
58. Koeppen BM: Electrophysiology of collecting duct H⁺ secretion: Effect of inhibitors. *Am J Physiol* 256: F79–F84, 1989
59. Greenlee MM, Lynch IJ, Gumz ML, Cain BD, Wingo CS: Mineralocorticoids stimulate the activity and expression of renal H⁺, K⁺-ATPases. *J Am Soc Nephrol* 22: 49–58, 2011
60. Stone DK, Seldin DW, Kokko JP, Jacobson HR: Mineralocorticoid modulation of rabbit medullary collecting duct acidification. A sodium-independent effect. *J Clin Invest* 72: 77–83, 1983
61. Wagner CA, Devuyst O, Bourgeois S, Mohebbi N: Regulated acid-base transport in the collecting duct. *Pflügers Arch* 458: 137–156, 2009
62. Wesson DE, Jo CH, Simoni J: Angiotensin II receptors mediate increased distal nephron acidification caused by acid retention. *Kidney Int* 82: 1184–1194, 2012
63. Wesson DE, Simoni J, Broglio K, Sheather S: Acid retention accompanies reduced GFR in humans and increases plasma levels of endothelin and aldosterone. *Am J Physiol Renal Physiol* 300: F830–F837, 2011
64. Al-Awqati Q: Terminal differentiation of intercalated cells: The role of hensen. *Annu Rev Physiol* 65: 567–583, 2003
65. Gao X, Eladari D, Leviel F, Tew BY, Miró-Julià C, Cheema FH, Miller L, Nelson R, Paunescu TG, McKee M, Brown D, Al-Awqati Q: Deletion of hensen/DMBT1 blocks conversion of beta- to alpha-intercalated cells and induces distal renal tubular acidosis. *Proc Natl Acad Sci U S A* 107: 21872–21877, 2010
66. Weiner ID, Hamm LL: Molecular mechanisms of renal ammonia transport. *Annu Rev Physiol* 69: 317–340, 2007
67. Tizianello A, Deferrari G, Garibotto G, Robaudo C, Bruzzone M, Passerone GC: Renal ammoniogenesis during the adaptation to metabolic acidosis in man. *Contrib Nephrol* 31: 40–46, 1982
68. Hamm LL, Simon EE: Ammonia transport in the proximal tubule. *Miner Electrolyte Metab* 16: 283–290, 1990
69. Kinsella JL, Aronson PS: Interaction of NH₄⁺ and Li⁺ with the renal microvillus membrane Na⁺-H⁺ exchanger. *Am J Physiol* 241: C220–C226, 1981
70. Nagami GT: Ammonia production and secretion by isolated perfused proximal tubule segments. *Miner Electrolyte Metab* 16: 259–263, 1990
71. Nagami GT: Enhanced ammonia secretion by proximal tubules from mice receiving NH₄Cl: Role of angiotensin II. *Am J Physiol Renal Physiol* 282: F472–F477, 2002
72. Houillier P, Bourgeois S: More actors in ammonia absorption by the thick ascending limb. *Am J Physiol Renal Physiol* 302: F293–F297, 2012
73. Weiner ID, Verlander JW: Renal ammonia metabolism and transport. *Compr Physiol* 3: 201–220, 2013
74. Kikeri D, Sun A, Zeidel ML, Hebert SC: Cell membranes impermeable to NH₃. *Nature* 339: 478–480, 1989
75. Heitman J, Agre P: A new face of the Rhesus antigen. *Nat Genet* 26: 258–259, 2000
76. Marini AM, Matassi G, Raynal V, André B, Cartron JP, Chérif-Zahar B: The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. *Nat Genet* 26: 341–344, 2000
77. Nakhoul NL, Abdulnour-Nakhoul SM, Boulpaep EL, Rabon E, Schmidt E, Hamm LL: Substrate specificity of Rhbg: Ammonium and methyl ammonium transport. *Am J Physiol Cell Physiol* 299: C695–C705, 2010
78. Nakhoul NL, Lee Hamm L: Characteristics of mammalian Rh glycoproteins (SLC42 transporters) and their role in acid-base transport. *Mol Aspects Med* 34: 629–637, 2013
79. Seldin DW, Rector FC Jr.: Symposium on acid-base homeostasis. The generation and maintenance of metabolic alkalosis. *Kidney Int* 1: 306–321, 1972
80. Schrier RW: Decreased effective blood volume in edematous disorders: What does this mean? *J Am Soc Nephrol* 18: 2028–2031, 2007
81. Gledhill N, Beirne GJ, Dempsey JA: Renal response to short-term hypocapnia in man. *Kidney Int* 8: 376–384, 1975
82. Madias NE, Adrogué HJ: Cross-talk between two organs: How the kidney responds to disruption of acid-base balance by the lung. *Nephron, Physiol* 93: 61–66, 2003
83. Polak A, Haynie GD, Hays RM, Schwartz WB: Effects of chronic hypercapnia on electrolyte and acid-base equilibrium. I. Adaptation. *J Clin Invest* 40: 1223–1237, 1961
84. Madias NE, Schwartz WB, Cohen JJ: The maladaptive renal response to secondary hypocapnia during chronic HCl acidosis in the dog. *J Clin Invest* 60: 1393–1401, 1977

Published online ahead of print. Publication date available at www.cjasn.org.