7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology

Asilomar Conference Grounds
Pacific Grove, California
September 18-22, 2011

www.the-aps.org/enac
### 2011 APS Conference

**7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology**

#### APS Council

<table>
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<tr>
<th>President</th>
<th>Past President</th>
<th>President-Elect</th>
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<tr>
<td>Joey P. Granger</td>
<td>Peter D. Wagner</td>
<td>Susan M. Barman</td>
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Kenneth M. Baldwin  
Ida Llewellyn-Smith  
Jane F. Reckelhoff  

David P. Brooks  
Patricia E. Molina  
Curt D. Sigmund  

Dennis Brown  
Usha Raj  
Alan F. Sved  

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Pamela K. Carmines  
Joseph R. Haywood  
Hershel Raff  

John C. Chatham  
Ronald M. Lynch  

Martin Frank  
Thomas A. Pressley  
Jeff M. Sands  

#### Conference Organizers

<table>
<thead>
<tr>
<th>Thomas R. Kleyman (Chair)</th>
<th>David Pearce (Co-Chair)</th>
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<tr>
<td>Univ. of Pittsburgh</td>
<td>Univ. of California, San Francisco</td>
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Marcelo Carattino  
Aniko Fejes-Toth  
Toshiro Fujita  

Univ. of Pittsburgh  
Dartmouth Med. Sch.  
Univ. of Tokyo, Japan  

Cathy Fuller  
John Funder  
Edith Hummler  

Univ. of Birmingham at Alabama  
Prince Henry’s Inst. of Med. Res., Australia  
Univ. of Lausanne, Switzerland  

Olivier Staub  
Gordon Williams  

Univ. of Lausanne, Switzerland  
Harvard Med. Sch.  

#### Acknowledgements

The Conference Organizers and The American Physiological Society gratefully recognize the generous financial support provided through unrestricted educational grants from:

Daiichi-Sankyo Company, Ltd.  
Dainippon Sumitomo Pharmaceuticals Company, Ltd.  
NIH, National Institute of Diabetes and Digestive and Kidney Diseases  
Amgen  
Otsuka  
Kent Scientific, Inc.
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<td>Keynote Lecture:</td>
<td>ENaC and Related</td>
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<td>Hypertension</td>
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<td>R. Lifton, Yale Univ.</td>
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<td>Symposium III (continued):</td>
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<td>Children, Toronto, Canada</td>
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<td>Poster Presentations</td>
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*2011 APS Conference*

**7th International Symposium on Aldosterone and the ENaC/DEGEPERF Family of Ion Channels: Molecular Mechanisms and Pathophysiology**

September 18—22, 2011,
Asilomar Conference Grounds, Pacific Grove, CA
GENERAL INFORMATION

Location:
The 2011 APS Conference: 7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology, will be held September 18—22, 2011, at the Asilomar Conference Grounds, 800 Asilomar Ave., Pacific Grove, CA 93950, telephone (831) 642-4222, FAX: (831) 642-4261.

On-Site Registration Information:
On-site registration will be available at the conference for badge pick-up, receipts, program distribution and conference information. The registration desk will be open daily in the Chapel Meeting room. If you are staying at an off-site hotel you will need to purchase meal tickets in order to dine with the other conference registrants. Meal tickets can be purchased at the front desk of the Heart Social Hall, located on the Asilomar Conference Grounds.

On-site Registration Hours:
Sunday, September 18.................3:00—9:00PM
Monday, September 19............7:00AM—6:00 PM
Tuesday, September 20...........7:00 AM—6:00 PM
Wednesday, September 21....7:00 AM—6:00 PM
Thursday, September 22........ 7:00—10:30 AM

On-Site Registration Fees:
On-site registration for this conference will not be available.

Press:
Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

Ancillary Session:
APS Career Workshop: This special session entitled: “Writing Your First Paper: The Ins and Outs of Authorship” will be presented by Thomas Schmidt, member of the APS Career Opportunities in Physiology Committee. Discuss the criteria for authorship and various roles authors can play during the research process and preparation and publication of a manuscript. Through case studies, explore real-life scenarios and how best to deal with the various issues that can arise with authorship.

Program Objective:
A major goal of this conference will be to bring together clinical and basic researchers from all over the globe with an interest in ENaC (and related transporters) and aldosterone, particularly as they pertain to renal and cardiovascular disorders, including hypertension. Hence, the scientific themes are a blend of clinical, basic and translational research, which allow a diverse community to break down obstacles to communication and engage in crosstalk, which would not otherwise be possible. By focusing on ENaC and aldosterone in the context of the renal and cardiovascular systems, the meeting provides a window into basic and clinical questions, which are fundamental to human biology in health and disease.

The conference program combines presentations from leading authorities with presentations from young investigators pursuing research on ENaC and aldosterone. Ample time for poster sessions will allow for in-depth discussions, and special abstract-based oral sessions will highlight ongoing research of students, fellows, and young scientists.

Target Audience:
This meeting is intended to bring together world leaders in clinical, translational and basic research in aldosterone and ENaC, and related areas for a multi-day intensive retreat to present and discuss the latest research in the field.

This meeting has been made possible through the generous support from:

Daiichi-Sankyo Company, Ltd.
Dainippon Sumitomo Pharmaceuticals, Company, Ltd.
NIH, National Institutes of Diabetes and Digestive and Kidney Diseases
Amgen
Otsuka
Kent Scientific, Inc.
**SUNDAY, SEPTEMBER 18, 2011**

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<th>Time</th>
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<tbody>
<tr>
<td>7:30 PM</td>
<td><strong>PLENARY LECTURE</strong>&lt;br&gt;1.0 Opening Comments. <strong>Thomas R. Kleyman.</strong> Univ. of Pittsburgh and <strong>David Pearce.</strong> Univ. of California, San Francisco.</td>
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<tr>
<td>7:40 PM</td>
<td><strong>Aldosterone and Heredity Hypertension.</strong> <strong>Richard Lifton.</strong> Yale Univ. Sch. of Med.</td>
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**MONDAY, SEPTEMBER 19, 2011**

**Symposia I 2.0 STRUCTURE AND FUNCTION OF ENaC AND RELATED TRANSPORTERS**<br>Mon., 8:00 AM-12:00 Noon, Chapel Room.<br>Chair: **Laurent Schild,** Univ. of Lausanne, Switzerland.

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<th>Time</th>
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<tr>
<td>8:00 AM</td>
<td><strong>ENaC Structure/function Overview.</strong> <strong>Laurent Schild.</strong> Univ. of Lausanne, Switzerland.</td>
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<tr>
<td>8:20 AM</td>
<td><strong>Regulation of ENaC Expression in Rat Kidney.</strong> <strong>Larry Palmer.</strong> Weill Med. Coll. of Cornell Univ.</td>
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<tr>
<td>8:40 AM</td>
<td><strong>ENaC Regulation in the Connecting Tubule.</strong> <strong>Johannes Loffing.</strong> Univ. of Zurich, Switzerland.</td>
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<td>9:00 AM</td>
<td><strong>Structural Insights into the Regulation of ENaCs by External Na.</strong> <strong>Shaohu Sheng.</strong> Univ. of Pittsburgh.</td>
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<td>9:20 AM</td>
<td><strong>Structural Transitions Associated with the Gating of ASIC1a.</strong> <strong>Marcelo Carattino.</strong> Univ. of Pittsburgh.</td>
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<td>9:40 AM</td>
<td><strong>Mechanisms of pH-Dependant Gating of ASICs.</strong> <strong>Stephen Kellenberger.</strong> Univ. of Lausanne, Switzerland.</td>
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<td>10:00 AM</td>
<td>Break.</td>
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<td>10:30 AM</td>
<td><strong>Mechanisms and Physiological Importance of ENaC Regulation by Growth Factors and Small GTPases.</strong> <strong>Alexander Staruschenko.</strong> Med. Coll. of Wisconsin.</td>
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<tr>
<td>11:10 AM</td>
<td><strong>A Synthetic Serine Protease Inhibitor Camostat Mesilate Inhibited the Proteolytic Activation of γENaC in the Kidney of Aldosterone-infused Rats.</strong> <strong>Kimio Tomita.</strong> Kumamoto Univ. Japan.</td>
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**Symposia II 3.0 STRUCTURE AND FUNCTION OF MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS**<br>Mon., 3:00-5:30 PM, Chapel Room.<br>Chair: **Peter Fuller,** Prince Henry's Inst. of Med. Res. Melbourne, Australia.

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<tr>
<td>3:00 PM</td>
<td><strong>Structure-Function Relationships in the Mineralocorticoid Receptor and Interactions with Novel Transcriptional Coregulators.</strong> <strong>Peter Fuller.</strong> Prince Henry's Inst. of Med. Res. Melbourne, Australia.</td>
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<tr>
<td>3:20 PM</td>
<td><strong>Physiologic Roles of MR Revealed by Tissue Selective Knockouts.</strong> <strong>Morag Young.</strong> Prince Henry's Inst. of Med. Res. Melbourne, Australia.</td>
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<td>3:40 PM</td>
<td><strong>Epithelial Sodium Channel (ENaC) is a Key Mediator of Growth Hormone-Induced Sodium Retention: Pathophysiology of Volume Expansion in Acromegalic Patients.</strong> <strong>Marc Lombes.</strong> INSERM, Paris, France.</td>
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<td>4:00 PM</td>
<td><strong>Molecular Mechanisms of Mineralocorticoid Receptor Function in Heart.</strong> <strong>Frederic Jaisser.</strong> INSERM, Paris, France.</td>
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<td>4:20 PM</td>
<td><strong>Subcellular Distribution of the Mineralocorticoid Receptor.</strong> <strong>Celso Gomez-Sanchez.</strong> Univ. of Mississippi, Jackson.</td>
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<td>4:40 PM</td>
<td><strong>Vascular Mineralocorticoid Receptors Mediate Aldosterone-Dependant Vascular Injury.</strong> <strong>Iris Jaffe.</strong> Tufts Univ. Sch. of Med.</td>
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<td>Renal Nedd4-2 is Crucial for NCC Regulation and Ca Balance</td>
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<td>4.0</td>
<td>A New Invertebrate Model for the Study of the Epithelial Sodium Channel (ENaC)</td>
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<td>xShroom1 Regulates the Number of ENaC Channels Inserted in the Membrane of Oocytes from Xenopus laevis</td>
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<td>6.0</td>
<td>Proteolytic Channel Activation by Plasmin Involves Two Distinct Cleavage Sites in the γ-Subunit of Human ENaC</td>
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<td>7.0</td>
<td>Does Insulin Regulate ENaC?</td>
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<td>8.0</td>
<td>Cleavage of Endogenous γENaC and Elevated Abundance of αENaC Is Associated with Increased Na+ Transport in Response to Apical Fluid Volume Expansion in Human 11441 Airway Epithelial Cells</td>
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<td>9.0</td>
<td>Aldosterone-Independent Regulation of ENaC in ADX Mice</td>
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<td>10.0</td>
<td>Collecting Duct-Specific Endothelin B Receptor Knockout Increases ENaC Activity</td>
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<td>Disease Causing Mutations Affect ENaC Gating</td>
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TUESDAY, SEPTEMBER 20, 2011

Symposia III

REGULATION OF ENaC BIOGENESIS, TRAFFICKING AND GATING

Tues., 8:00 AM-12:00 Noon, Chapel Room.

Chair: Douglas Eaton, Emory Univ.

8:00 AM

5.1 Overview of ENaC Regulation. Douglas Eaton, Emory Univ.

5.2 CNK3 and the ENaC Regulatory Complex. Rama Soundararajan, Univ. of California, San Francisco.

8:40 AM

5.3 Role of Purinergic Signaling Regulating ENaC Activity. James Stockand, UTHSCSA.

9:00 AM

5.4 MicroRNAs: Novel ENaC Regulators. Michael Butterworth, Univ. of Pittsburgh.

9:20 AM

5.5 Interplay Between Kinases, Nedd4-2 and ENaC. Kenneth Hallows, Univ. of Pittsburgh.

9:40 AM

5.6 P2Y2-R Regulation of ENaC-mediated Na⁺ Absorption in Airway Epithelia. Jack Stutts, Univ. of North Carolina, Chapel Hill.

10:00 AM Break

10:30 AM

5.7 Ubiquitylation-deubiquitylation Cycles in the Control of Membrane Protein Stability and Trafficking. Olivier Staub, Univ. of Lausanne, Switzerland.

10:50 AM

5.8 Regulation of NCC by the Aldosterone-SGK1-NEDD4-2 Pathway. Dagmara Lagnaz, Univ. of Lausanne, Switzerland.

11:10 AM

5.9 ENaC Regulation by Proteases. Christoph Korbmacher, Univ. Erlangen-Nürnberg, Germany.

1:00 PM

5.10 Deletion of the Ubiquitin Ligase NeddHL in Lung Epithelia Causes Cystic Fibrosis-like Disease. Daniela Rotin, Hosp. for Sick Children, Toronto, Canada.

1:20 PM

5.11 Regulation of Ubiquitin Ligase Activity and Phosphorylation by SGK1. Vivek Bhalla, Stanford Univ.

1:40 PM


1:52 PM

5.13 Rab-GAP Regulation of Epithelial Sodium Channel (ENaC) Forward Trafficking in Response to Aldosterone and Vasopressin. Xiubin Liang, Univ. of Pittsburgh.

2:04 PM

5.14 The Role of mTOR and SGK1 in Mediating Aldosterone Regulation of ENaC in vivo. Atif Kidwai, Univ. of California, San Francisco.

2:30 PM Break

Symposia IV

ALDOSTERONE: SYNTHESIS CROSSTALK AND NON-EPITHELIAL ACTIONS

Tues., 3:00-4:30 PM, Chapel Room.

Chair: William Rainey, Georgia Hlth. Sci. Univ.

3:00 PM


3:20 PM


3:40 PM


4:00 PM

6.4 Compartment-Specific Mineralocorticoid Receptor Signaling. Claudia Grassmann, Univ. Halle-Wittenberg, Germany.

4:20 PM Break

PLENARY LECTURE

Tues., 4:30-5:30 PM, Chapel Room.
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<td>11</td>
<td>Dissection of the Aldosterone and Glucocorticoid-dependent Pathway Implicated in Sodium Retention in the Rat. V. Ponce de Leon, J. Canonica and E. Hummler. Univ. of Lausanne, Switzerland.</td>
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<td>15</td>
<td>The Role of Renal Mineralocorticoid Versus Glucocorticoid Receptor in Oedematous Diseases. J. Canonica, V. Ponce de Leon, F. Frey and E. Hummler. Univ. of Lausanne, Switzerland and Univ. of Bern, Switzerland.</td>
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<td>17</td>
<td>Modulation of the Epithelial Sodium Channel (ENaC) Activity by Noradrenaline in Cultured Collecting Duct Cells is Partially Mediated by α₂-Adrenoceptors. M. Mansley, M. Bertog and C. Körbacher. Friedrich-Alexander-Univ. Erlangen-Nürnberg, Germany.</td>
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<td>19</td>
<td>Identification of Permissive Insertion Sites for Generating Functional Fluorescent Mineralocorticoid Receptors. D. Alvarez de la Rosa, C. Aguilar, I. Hernandez-Diaz, F. Lorenzo-</td>
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10:47 AM 10.9 Energetic and Structural Basis for Activation of ENaC by CAP-3. Pradeep Kota. Univ. of North Carolina, Chapel Hill.

10:58 AM 10.10 Aldosterone is Dispensable for Renal but not for Colonic Regulation of Potassium Homoeostasis. Abhijeet Todkar. Univ. of Zurich, Switzerland.


11:20 AM 10.12 A Novel Redox-sensitive E3-Ubiquitin Ligase Regulates Surface Expression of Epithelial Sodium Channels in Alveolar Epithelial Cells. Amrita Kumar. Emory Univ.

11:32 AM 10.13 NADPH Oxidase and ENaC. My Helms. Emory Univ.


Career Workshop

11.0 THE INS AND OUTS OF AUTHORSHIP
Wednes., 3:00-4:00 PM, Chapel Room.

Chair: Thomas Schmidt, Univ. of Iowa.

3:00 PM 11.1 The Ins and Outs of Authorship. Thomas Schmidt. Univ. of Iowa.

Symposia VI

12.0 CONGESTIVE HEART FAILURE: THE INTERTWINED ROLES OF WATER AND SALT
Wednes., 4:00-5:30 PM, Chapel Room.

Chair: David Pearce, Univ. of California, San Francisco.

4:00 PM 12.1 Potential Future Role of Mineralocorticoid Receptor Blockade in Patients with Heart Failure. Bertram Pitt. Univ. of Michigan.

4:25 PM 12.2 Role of Vasopressin in the Water Retention in Congestive Heart Failure-Pathogenesis and Treatment. Tomas Berti. Univ. of Colorado, Denver.

Plenary Lecture

13.0 PLEINARY LECTURE
Wednes., 5:00-6:00 PM, Chapel Room.

5:00 PM 13.1 ASIC Structure and Function. Michael Welsh. Univ. of Iowa, HHMI.

THURSDAY, SEPTEMBER 22, 2011

Symposia VII

14.0 ENaC PATHOPHYSIOLOGY
Thurs., 8:00-10:00 AM, Chapel Room.

Chair: Bernard C. Rossier, Univ. of Lausanne, Switzerland.
2011 APS Conference
7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology

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3.0 Structure and Function of Mineralocorticoid and Glucocorticoid Receptors........14
4.0 ENaC Structure and Regulation............................................................................15
5.0 Regulation of ENaC Biogenesis, Trafficking and Gating.................................19
6.0 Aldosterone: Synthesis Crosstalk and Non-Epithelial Actions.........................21
7.0 Plenary Lecture..................................................................................................21
8.0 Aldosterone and ENaC.....................................................................................22
9.0 Remembering J. D. Horisberger and D. J. Benos.............................................26
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14.0 ENaC Pathphysiology....................................................................................28
15.0 Aldosterone Pathphysiology...........................................................................29

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2.2 REGULATION OF ENaC EXPRESSION IN RAT KIDNEY

Lawrence Palmer1, Gustavo Frindt1


The activity of epithelial Na channels (ENaC) in the apical membrane of the CCD and CNT is strongly and inversely dependent on dietary Na, an effect mediated at least in part by the mineralocorticoid aldosterone. The surface expression of cleaved forms of the ENaC subunit was the biochemical parameter best correlated with channel function. Even under conditions of high-salt intake, with no measurable channel activity, significant expression of αENaC and uncleaved γENaC were observed at the surface of the cell. We further examined the nature of this expression by the deglycytyruting enzymes PNGaseF and endoH. Cleaved forms of γENaC at the surface were sensitive to PNGaseF but not endoH, indicating that these forms were fully processed on their way to the surface. In contrast, the full-length form of γENaC was sensitive to endoH, suggesting that it was incompletely processed. In salt-replete rats, much of the γENaC at the surface was also endoH sensitive. However in salt-depleted animals much of the γENaC became endoH resistant. The increase in endoH-resistant γENaC at the cell surface correlated well with that of cleaved, endoH-resistant γENaC. We conclude that ENaC can reach the surface in both mature and immature forms, possibly involving two routes to the apical membrane. The mature form of the protein represents the fully active channel, and its delivery to the membrane is stimulated by the Na depletion. NII DK59609. Reference: Frindt, G, Ergonol, Z. and Palmer, L.G. Surface expression of epithelial Na channel protein in rat kidney. J. Gen. Physiol. 131:617-627 (2008).

2.3 ENaC REGULATION IN THE CONNECTING TUBULE

Sukesh Loffing1

1.Toke of Anatomy, Univ. of Zurich, Winterthurerstrasse 190, Zurich, 8057, Switzerland.

The renal connecting tubule (CNT) is part of the aldosterone-sensitive distal nephron (ASDN) and connects the late distal convoluted tubule (DCT2) with the collecting duct (CD). A variety of recent ion transport, electrophysiological and morphological studies on various animal models were fully processed on their way to the surface. In contrast, the full-length form of ENaC/DEG member, ASIC1, was sensitive to endoH, suggesting that it was incompletely processed. In salt-replete rats, much of the γENaC at the surface was also endoH sensitive. However in salt-depleted animals much of the γENaC became endoH resistant. The increase in endoH-resistant γENaC at the cell surface correlated well with that of cleaved, endoH-resistant γENaC. We conclude that ENaC can reach the surface in both mature and immature forms, possibly involving two routes to the apical membrane. The mature form of the protein represents the fully active channel, and its delivery to the membrane is stimulated by the Na depletion. NII DK59609. Reference: Frindt, G, Ergonol, Z. and Palmer, L.G. Surface expression of epithelial Na channel protein in rat kidney. J. Gen. Physiol. 131:617-627 (2008).

2.4 STRUCTURAL INSIGHTS INTO THE REGULATION OF ENaCS BY EXTERNAL pH

Shahid Shergill1

1.Medicine, Univ. of Pittsburgh, 5829 Scale Hall, 3550 Terrace St., Pittsburgh, PA, 15261.

High concentrations of extracellular Na+ reduce ENaC Po via a mechanism referred to as Na+ self-inhibition. Many extracellular factors including cations, anions, proteases, temperature and small molecules regulate ENaC Po, in part, by modulating the Na+ self-inhibition response. The N-terminus ectodomain expression system has provided a convenient tool to characterize and probe the structural basis of Na+ self-inhibition. The crystal structure of an ENaC/DEG member, ASIC1, also provides an invaluable tool to interpret results from mutagenesis studies and to develop hypotheses that drive future studies. The structural basis of Na+ self-inhibition remains unclear. However, site-directed mutagenesis studies have implicated several extracellular structures in Na+ self-inhibition, including the finger, thumb and palm subdomains, as well as the pore domains. While all cloned ENaCs exhibit Na+ self-inhibition, the three ENaC subunits appear to have distinct roles in the process with the γ subunit being the most important. We speculate that Na+ self-inhibition serves as a central mechanism regulating the efficacies of specific channel regulators. Elucidating the allosteric mechanism of Na+ self-inhibition may provide clues for development of novel therapeutic agents. (Support: NHI R01 ES01470, R01 DK054354, and P30 DK079370). Reference: Warnaco KL, Sheng N, Chan J, Kleyman TR, Sheng S. Extracellular allosteric regulatory subdomain within the gamma subunit of the epithelial Na channel (ENaC). J. Physiol. 449:111-35 (2009).

2.5 STRUCTURAL TRANSITIONS ASSOCIATED WITH THE GATING OF ASIC1a Mucolipin Catalase

Depart Med, Univ. of Pittsburgh, 3550 Terrace St., S828 Scale Hall, Pittsburgh, PA, 15261.

Acid-sensing ion channels are trimeric proton-gated cation selective channels expressed in the nervous system. Proton binding to the extracellular region of these channels triggers activation. Residues in the TM2 helices define the ion selective properties of ASIC1a. We conclude that ENaC can reach the surface in both mature and immature forms, possibly involving two routes to the apical membrane. The mature form of the protein represents the fully active channel, and its delivery to the membrane is stimulated by the Na depletion. NII DK59609. Reference: Frindt, G, Ergonol, Z. and Palmer, L.G. Surface expression of epithelial Na channel protein in rat kidney. J. Gen. Physiol. 131:617-627 (2008).

2.6 MECHANISMS OF pH-DEPENDENT GATING OF ASICs

Stefan Kellenberger1

1.Dpt. of Pharmacology and Toxicology, Univ. of Lausanne, Rue du Bugnon 27, Lausanne, Switzerland.

ASICs are activated by a lowing of the extracellular pH. Initial analysis of the crystal structure suggested an acidic pocket in the thumb domain as a candidate sensor. In this study, we determined whether other parts of the channel protein also contribute to pH sensing in ASIC1a we have applied a systematic approach. We calculated the pKa of all extracellular His, Glu and Asp residues using a Poison-Boltzmann continuum approach based on the ASIC 3D structure. The role of residues with a pKa in the pH range of ASIC gating was then assessed by site-directed mutagenesis and functional analysis. The localization of putative pH-sensing residues suggests that pH changes control ASIC gating by protonation / deprotonation of many residues per subunit in different channel domains, rather than protons acting differently from larger ligands which bind to a single number of distinct binding sites. We speculate that these putative pH-sensing residues participate in both activation and inactivation. Analysis of the function of residues in the palm domain close to the central vertical axis of the channel allowed for prediction of conformational changes of this region during gating. Based on this work and on studies by other groups we conclude that different domains contribute to pH-dependent gating of ASICs, with the thumb and the lower palm domains playing likely the most important roles. The parts of the protein that link the pH-sensing domains to the channel gate are also critical for ASIC function and may be additional potential drug target sites. (Swiss NSF grant 310030_135542).

2.7 MECHANISMS AND PHYSIOLOGICAL IMPORTANCE OF ENaC REGULATION BY GROWTH FACTORS AND SMALL GTPASES

Alexander Staschke1


Long term control of blood pressure involves Na+ homeostasis through the precise regulation of ENaC in the ASDN. EGF and related EGF family members bind to their specific receptors as acting as growth factors for renal development, physiology and pathophysiology. Under physiological conditions, ErbB receptors play an important role in the regulation of renal hemodynamics and electrolyte handling by the kidney in different physiological or pathophysiological states. ErbB activation may mediate either beneficial or detrimental effects on the kidney. Stimulation of ErbB receptors activates an intracellular cascade involving small GTPases, particularly Rac1. Small G proteins and their regulatory proteins contribute to the pathology of renal and cardiovascular diseases. We demonstrate that ENaC is regulated by EGF and Rac1, possibly through a convergent mechanism. Dihl-salt-sensitive (SS) rats used in these studies develop severe hypertension on high-salt diet. We provide data indicating that ENaC contributes to the development of hypertension in the SS rat strain. Furthermore, our data reveal that EGF concentration is reduced in the SS rats, which we hypothesize may affect ENaC activity. We speculate that EGF acting through Rac1 is important for physiological control of renal sodium handling through regulation of ENaC. REFERENCES: 1) Karpuschov A, Levenchenko V, Itatoyan D, Pavlov T, Staschke A. (2011) Novel role of Rac1/WAVE signaling mechanism in regulation of the epithelial Na channel (ENaC). Hypertension 57:996-1002. 2) Levenchenko V, Zhelova NA, Staschke A, Winkler TS, Vandewalle X, Wilson PD, Staschke A. (2010) EGF and its related growth factors increase sodium transport in mpkCCD cells via ErbB2 (ras-HIR-2) receptor. J. Cell Physiol. 225(1):252-259.

2.8 HYPOTONICITY-INDUCED UPREGULATION OF β- AND γENaC EXPRESSION THROUGH SUPPRESSION OF ERK BY INDUCTING MKP-1

Yoshiharu Manzuka1, Naomi Niisato1, Mariko Ohita1

1.Dpt. of Molecular Cell Physiology, Kyoto Prefectural Univ. of Med., Kamigyo-ku, Kyoto, 602-8566, Japan. Institute for Food Educ. and HIIB, St. Agnes’ Univ., Kyoto 602-8015, Japan. We studied a physiological role of ERK in stimulatory action of hypotonicity on ENaC-mediated Na+ reabsorption in renal epithelial A6 cells, and obtained the following observations: 1) Hypotonicity dephosphorylated ERK after transient phosphorylation; 2) PD98059 (a MEK inhibitor) dephosphorylating ERK enhanced the stimulatory action of hypotonicity on ENaC expression of both β- and γENaC; 3) Hypotonicity increased expression of MKP-1 mRNA by activating p38, while inhibition of MKP-1 by NSC95397 (an MKP-1 inhibitor) suppressed the dephosphorylation of ERK; 4) Inhibition of p38 suppressed MKP-1 induction, preventing hypotonicity from dephosphorylating ERK. We speculate that MKP-1 induction by hypotonicity in ENaC-mediated Na+ reabsorption in renal epithelial A6 cells.
2.9 A SYNTHETIC SERINE PROTEASE INHIBITOR CAMOSTAT MESILATE INHIBITED THE PROTEOLYTIC ACTIVATION OF αENaC IN THE KIDNEY OF ALDOSTERONE-INFUSED RATS
Kimio Tomita1
1Dept. of Nephrology, Kramaton Univ., Honjo 1-1-1, Kramaton, 860-8556, Japan.
ENaC consists of α, β, and γ subunits, and the activation of ENaC is mainly regulated by aldosterone in living body. It was reported that aldosterone induced a molecular weight shift of αENaC from 65 to 70 kDa, and recently this shift has been considered as the result of proteolytic cleavages by serine proteases and necessary for the activation of ENaC from the in vitro experiment. But detail mechanisms about the cleavage of γENaC in vivo are still unclear. In order to study the role of enalaprilase in this cleavage in vivo, we administrated a synthetic serine protease inhibitor camostat mesilate to aldosterone-infused rats. Camostat decreased 75kDa form of γENaC and produced the new about 75kDa form with increase of urinary Na/K ratio, suggesting that camostat inhibited one site of the dual cleavages of γENaC and suppressed the activation of ENaC. Prostasin is one candidate serine protease involved in the cleavage of γENaC in these model rats, because prostasin was shown to cleave this subunit in vitro and its expression in mouse skin. Nature Comm. 2011, 2: 161.

2.10 LESSONS LEARNED FROM KNOCKOUT STUDIES
Edith Hammerle1
1Dept. of Pharmacology & Toxicology, Univ. of Lausanne, Bagnon 27, Lausanne, 1055, Switzerland.
The so-called epithelial Na+ channel (ENaC) is an important modulator of Na+ homeostasis, and thereby plays a critical role in regulating blood pressure, renal function and fluid balance in the lung. ENaC distribution is widespread and has been found in a variety of epithelia, including sweat glands, epidermis and taste cells. Mice lacking ENaC expression die soon after birth due to failure to clear the lungs of liquid, but also show severe skin dehydration. Furthermore, these animals present metabolic acidosis with lower blood pH and low bicarbonate concentrations, suggesting a metabolic component added to the probable respiratory acidosis. Tissue-specific and inducible knockout mice are valuable tools to validate and to define the importance of pathways and proteins that are implicated in ENaC-mediated Na+ transport and blood pressure regulation. The approach is also useful for candidate gene identification but has been identified so far only in cell culture systems, like some cell channel expressing proteases. We recently identified CAPI Prox1 as upstream activator of the protease-activated receptor 2 that may play an important role in regulation of ENaC absorption in the kidney. Transgenic mice lacking CAPI Prox1 expression in mouse skin will help to define the role in the kidney and other tissue and organs. They serve as mammalian models of human diseases and later on to validate drug targets. References: Frattoli S, Camerer E, Cisante G, Risser M, Menbrez M, Charles R-P, Beermann F, Stehle J-C, Breiden B, Sandhoff K, Rottman S, Hadlak M, Wilson A, Ryser S, Steinhoff M, Coughlin S, Hummerl E, PAR2 absence completely rescues inflammation and inflammation caused by altered CAPI-Prox1 expression in mouse skin. Nature Commun. 2011, 2: 161.

2.11 CONFORMATIONAL TRAPPING OF THE CLOSED STATE OF ENaC
Ossama Kashlan1
1Medicine, Univ. of Pittsburgh, 3550 Terrace St., Pittsburgh, PA, 15261.
Atypical ion channels, the epithelial Na+ channel (ENaC) is proteolytically processed during biogenesis. ENaC is assembled from three homologous subunits, and it is the alpha and gamma domain are susceptible to proteolysis. Cleavage at two sites in either subunit releases an inhibitory tract and activates the channel. Peptides corresponding to either released end of the inhibitory peptide. We hypothesized that we could covalently bind the peptide to the channel after introducing Cys to define their role in the kidney and other tissue and organs. They serve as mammalian models of human diseases and later on to validate drug targets. References: Frattoli S, Camerer E, Cisante G, Risser M, Menbrez M, Charles R-P, Beermann F, Stehle J-C, Breiden B, Sandhoff K, Rottman S, Hadlak M, Wilson A, Ryser S, Steinhoff M, Coughlin S, Hummerl E, PAR2 absence completely rescues inflammation and inflammation caused by altered CAPI-Prox1 expression in mouse skin. Nature Commun. 2011, 2: 161.

2.12 THE EXTRACELLULAR DOMAIN OF ENaC IS A SENSOR OF THE EXTRACELLULAR MILIEU
Peter Snyder1, Daniel Coller1
1Tat, Mass. Molecular Physiology & Biophysics, Univ. of Iowa, 371 EMBR, Iowa City, IA, 52242.
The epithelial Na+ channel (ENaC) functions as a pathway for Na+ absorption across epithelia of the kidney and lung. In order to maintain homeostasis, ENaC activity must vary over a wide range. In Xenopus oocytes, ENaC activity increases in response to Na+ reabsorption. Conversely, ENaC activity is reduced in response to volume excess. Under these conditions, ENaC is exposed to extreme changes in extracellular ion concentrations. For example, Na+ and Ci range from 1-150 mM. pH can vary from 4.5-8 in response to metabolic acidosis and alkalosis as well as with changes in diet and volume status. Extracellular Na+ is known to inhibit ENaC through a process known as Na+ self-inhibition. We found that protons increase ENaC activity by reducing Na+ self-inhibition. This occurs through the titration of residues in the extracellular domains of β- and γENaC. Extracellular Ci inhibits ENaC, but the underlying mechanism has not been fully understood. Together, the data support a model in which the large highly structured extracellular domain functions as a sensor to modulate ENaC activity to respond to extrinsic changes in its environment. Support: HL-072256 (NIB) to PMS and 10PR2610282 (AHA) to DMC.

2.13 THE EPILEPTIC CHAPERONE, LHS1/GPR170, PLAYS A UNIQUE ROLE IN THE BIOGENESIS OF THE EPITHELIAL SODIUM CHANNEL
Teresa Back1, Lindsay Pjeschak1, Osamu Kashlan1, Thomas Kleyman2, Jeffrey Brodsky2
1Biological Sci, Univ. of Pittsburgh, 4259 5th Ave, Pittsburgh, PA, 15260. 2Med, Renal-Electrolyte Div, Univ of Pittsburgh, 3550 Terrace St, Pittsburgh, PA, 15261.
The Epithelial Sodium Channel (ENaC) is composed of three homologous subunits—α, β, and γ—that assemble in the endoplasmic reticulum (ER) to form the mature ENaC channel. However, heterotrimer formation is highly inefficient, thus targeting the channel subunits for Endoplasmic Reticulum Associated Degradation (ERAD). To characterize the mechanism of ENaC degradation, we used an ENaC expression system developed. Using this system, we found that Jem1 and Sgl1, two ER luminal Hsp40 chaperones, play an essential role in ENaC degradation. Expression of the human Hsp40 homologs similarly increased ENaC degradation in a Xenopus oocyte expression system. Jem1 and Sgl1 facilitate the ATP hydrolysis of the ER chaperone Hsc70/70 chaperone, BiP, although surprisingly BiP does not play a role in ENaC degradation. Therefore, we hypothesized that another ER chaperone with a Hsp70-like domain, Lhs1, may be involved. We found that Lhs1 is involved in the ENaC subunit degradation, but not the degradation of the β- or γ-ENaC subunits. Surprisingly, Lhs1 ATPase activity is dispensable for ENaC degradation. This result further supports our conclusion that the BiP chaperones, Jem1, Sgl1 and Lhs1, are acting in a BiP independent fashion. Preliminary data indicate the human lung homolog, GPR170, also affects ENaC biogenesis in oocytes. In conclusion, we have identified a new class of ER chaperones as novel effectors of ENaC degradation. Funded in part by K01DK090195 to T.M.B. and DK79307 to J.L.B. and T.R.K.
4.2. STRUCTURE-FUNCTION RELATIONSHIPS IN THE MINERALOCORTICOID GLUCOCORTICOID RECEPTORS

3.1 STRUCTURE-FUNCTION RELATIONSHIPS IN THE MINERALOCORTICOID RECEPTOR AND INTERACTIONS WITH NOVEL TRANSCRIPTIONAL COREGULATORS

3.2 PHYSIOLOGIC ROLES OF MR REVEALED BY TISSUE SELECTIVE KNOCKOUTS

3.3 EPISTEMAL SODIUM CHANNEL (ENaC) IS A KEY MEDIATOR OF GROWTH HORMONE (GH)-INDUCED SODIUM RETENTION. PATHOPHYSIOLOGY OF VOLUME EXPANSION IN ACRO-MEGALIC PATIENTS

Molecular Mechanisms and Pathophysiology

ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

Daniel Collier1, Peter Snyder2

Molecular Physiology & Biophysics and Int. Med., Univ. of Iowa, Carver Coll. of Med., 371 EMRB, Iowa City, IA, 52242.

A growing body of evidence suggests that the extracellular domain of ENaC functions as a sensor or in some cases directly interacts in response to changes in the extracellular environment. We previously demonstrated that it has dual effects on ENaC activity; they increase ENaC activity by decreasing Na+ self-inhibition, and inhibit ENaC activity by increasing CT inhibition. In the current work, we investigated the mechanisms by which it stimulates ENaC. We found that ENaC activation by NaCl is less sensitive to Na+ therefore, an effect mediated by the Na+ subunit. Our strategy was to mutate acidic residues in the extracellular domain of human ENaC that are conserved in rat γENaC. We expressed the mutant γENaC cDNAs (with wild type α- and βENaC) in Xenopus oocytes and tested the effect of changes on amiloride-sensitive Na+ current (by TEVC at -60 mV). We identified a group of 7 residues in the extracellular domain of ENaC (D164, Q165, D166, E292, E335, H439 and E455) that, when individually mutated to Ala, decreased Na+ activation of ENaC. Intriguingly, mutating the residues equivalent to E455 in αENaC (E477) and βENaC (E484) increased and decreased the response to acidic pH, respectively. Combining these seven mutations in βENaC with E484A generated a channel that was not activated by acidic pH. The data demonstrate that residues in humans β- and γENaC are required for regulation by pH. Supported by NIH HL072256 (PMS) and AHA 10HP261028 (DMC).

Marc Lorente1,2

1Molecular Physiology & Biophysics and Int. Med., Univ. of Iowa, Carver Coll. of Med., 371 EMRB, Iowa City, IA, 52242.

VOLUME EXPANSION IN ACRO-MEGALIC PATIENTS


GH excess in acromegalic patients is associated with volume expansion and hypertension but the underlying mechanisms remain unclear. We provided experimental evidence that GH elicits a direct vasopressor effect on ENaC-dependent Na transport (Kanemura, Endocrinology 2008). Renal metabolic studies performed on acromegalic rats revealed a decreased natriuretic response to furosemide compared to controls, whereas ammonium induced increased natriuresis. We showed an enhanced clearance of ouabain of ENaC and an increased abundance of ENaC in GC rats. The presence of functional GH receptors coupled to JAK/STAT and ERK activation was demonstrated in cortical collecting duct KCa3.1AC1 cells. GH-stimulated Na reabsorption, inhibited by GH antagonist, was associated with a GH-induced ouabain excretion. Natriuretic and kaliuretic responses to diuretics were compared in patients before and after acromegaly treatment (Clinicaltrials NCT00319008). Noradrenaline was more increased by ammonium in patients than before after acromegaly treatment (13.9 vs 6.3), while Na/Am ratio after furosemide was lower in untreated patients (5.2 vs 7.1). Ammonium-sensi
tive renal potential was also significantly higher before acromegaly treatment (53.8 vs 42 mV). Our findings suggest enhanced renal and extrarenal ENaC activity in acromegaly and provide first evidence that GH stimulates ENaC-mediated Na transport, contributing to salt and water swelling and high blood pressure in acromegalic patients. Support: Insrmed, Univ-Paris-Sud, AHP (CRC0062).

3.4 MOLECULAR MECHANISMS OF MINERALOCORTICOID RECEPTOR DEFECTS AND THEIR CLINICAL RELEVANCE

Frederic Jaisser

1Steroid Receptor Biology Group, Prince Henry’s Inst. of Med. Res., PO Box 5152, Clayton, 3168, Australia.

The mineralocorticoid (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol. In epithelial tissues, aldosterone selectivity is determined by the activity of 11β-hydroxysteroid dehydrogenase type II. In other tissues, including the heart and CNS, cortisol is the primary ligand for the MR; in some tissues cortisol is an antagonist. To understand the structural determinants of tissue and ligand-specific MR activation we have focused on interactions of the ligand binding domain (LBD) with the N-terminal domain and with putative coregulatory molecules. Both agonist and antagonist binding has been characterised using chimeras between the human (h)MR LBD and both the glucocorticoid receptor (GR) and the zebrafish (z)MR together with molecular modelling. An interaction between the N-terminus and C-terminus/LBD (N/C-interaction) observed in the MR is aldosterone-dependent but is unexpectedly antagonised by cortisol and DOC in the hMR but not the zMR. Nuclear receptor mediated transcription is critically dependent on, and modulated by, co-regulatory molecules. Yeast-2-hybrid screens with the MR LBD have identified proteins which interact in the presence of either aldosterone or cortisol but not both. These have been confirmed as coactivators of the full-length hMR in a transcription assay. The successful identification of ligand-specific interactions of the MR may provide the basis for the development of novel MR ligands with tissue specificity. Support: NHMRC 1002539.

3.5 SUBCELLULAR DISTRIBUTION OF THE MINERALOCORTICOID RECEPTOR

Celso Gomez-Sanchez1,2

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The mineralocorticoid receptor (MR) is a ligand-activated transcription factor in the steroid receptor superfamily. In the unbound state it is primarily in the cytosol; upon binding it enters the nucleus and binds hormone response elements to activate gene transcription. Translocation between the cytosol and nucleus involves binding to multiple chaperones. The MR also mediates rapid non-genomic actions. A panel of monoclonal antibodies against different epitopes of the MR has been developed. We performed subcellular fractionation by differential ultracentrifugation and density gradient purification and detection by western blot of M1 cells expressing the MR and of rat heart and hippocampus and used different antibodies to detect the MR. The MR was found in the cytosol, nuclei, mitochondria and microsomes. Using a combination of biotin labelling, loose grained centrifugation, and cationized albumin-dimethylamine-lysine conjugation of the plasma membrane in association with caveolin 1. MR were also demonstrated by immunoelectron microscopy in mitochondria of rat kidney (NHMDL27255 and VA) Ref: Gomez-Sanchez, C.E., de Rodriguez-A, A.F., Romero, D.G., Estes, J., Warder, M.P., Gomez-Sanchez, M.T., and Gomez-Sanchez, E.P. 2006. Development of a panel of monoclonal antibodies against the mineralocorticoid receptor. Endocrinology 147:1343-1348. Galimard, M.G., Erleine, A.G., Monte, M., Gomez-Sanchez, C., and Pwien-Pilipuk, G. 2010. The hsp90-FKB12 Complex Links the Mineralocorticoid Receptor to Motor Proteins and Persists Bound to the Receptor in Early Nuclear Events. Mol Cell Biol 30:1285-1299.

3.6 FKBP52 COMPLEX LINKS THE MINERALOCORTICOID RECEPTOR TO MOTOR PROTEINS AND PERSISTS BOUNDED TO THE RECEPTOR IN EARLY NUCLEAR EVENTS.

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Aldosterone and furosemide (Furo) regulate blood pressure by activating renal mineralocorticoid receptors (MR). In clinical trials MR antagonists decrease mortality and vascular ischemia out of proportion to modest decreases in bp, suggesting direct vascular protective effects of MR antagonists. We have found that aldosterone and Furo in human vascular smooth muscle cells (HSMC) and human aortic macrophage (HuAM) is hypertrophied and hypothesized that VSMC MR regulates genes that promote vascular injury. Using gene expression profiling, we identified the ratonogenic factor, placental growth factor (PGF), as a vascular MR target gene. In mouse vessels with endothelial damage and in human aortic smooth muscle cells, MIO, we found aldosterone and Furo effects similar to those in HuAM. Moreover, treatment of diseased human vessels with MR antagonist, decreases vascular PGF expression. In the mouse carotid injury model, ald-stimulated SMC proliferation and fibrosis observed in WT mice is inhibited in PGF KO mice. We recently developed a mouse model with...
SODIUM SELECTION OF AMILORIDE-SENSITIVE CURRENTS IN INNER EAR EPITHELIAL CELLS

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Luminal Na is maintained at 124 ± 0.001 mM in the oocytes expressing αβγENaC, which are contaminated with 150 mM NaCl and 150 mM KCl. In contrast, αβγENaCmediated Na permeability was increased to 42 ± 0.001 µS with xShroom1 sense and antisense respectively (n=18). The same results were obtained in oocytes expressing a DEG mutant αβγENaC (0.5±10 µS and 0.8±0.2 µS for oocytes injected with mRNA encoding a DEG mutant αβγENaC) or a DEG mutant αβγENaC (2.0±0.1 µS and 2.0±0.2 µS for oocytes injected with mRNA encoding a DEG mutant αβγENaC). These results suggest that the αβγENaC-mediated Na permeability is increased in the oocytes expressing xShroom1 sense and antisense respectively (n=18).

4.2. Molecular Mechanisms and Pathophysiology

ABSTRACTS OF INVITED AND VOLUNTARY PRESENTATIONS

INACTIVE ENaC STRUCTURE AND REGULATION

4.1

RENAL NEDD4-2 IS CRUCIAL FOR NCC REGULATION AND CA BALANCE

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To maintain proper Na/K balance and blood pressure (BP), aldosterone acts in the kidney by preventing ENaC degradation by the ubiquitin ligase Nedd4-2 (N4-2). To determine the role of N4-2 in Na+ reabsorption in the distal renal tubule [2]. In mouse receiving 50 µg aldosterone/kg body weight/24 hrs for 7 days as compared to vehicle treated mutants. Plasma aldosterone was increased under standard and high-Na diet conditions. The results suggest that the direct effect of N4-2 on b/gENaC in vivo, a compensatory regulation of b/gENaC, NCC and ROMK protein were increased, but aENaC protein and mRNA were decreased, suggesting decreased αβγENaC activity. The increased mRNA of NCC and ROMK, but decreased aENaC protein and mRNA are consistent with the results obtained in the mouse experiments.

N4-2 is completely lost in the different renal tubular segments in doxycycline-treated mutants. Plasma aldosterone was increased under standard and high-Na diet conditions. The results suggest that the direct effect of N4-2 on b/gENaC in vivo, a compensatory regulation of b/gENaC, NCC and ROMK protein were increased, but aENaC protein and mRNA were decreased, suggesting decreased αβγENaC activity. The increased mRNA of NCC and ROMK, but decreased aENaC protein and mRNA are consistent with the results obtained in the mouse experiments.

1.80 ± .50 µS with xShroom1 sense and antisense respectively (n=18). The same results were obtained in oocytes expressing a DEG mutant αβγENaC (0.5±10 µS and 0.8±0.2 µS for oocytes injected with mRNA encoding a DEG mutant αβγENaC) or a DEG mutant αβγENaC (2.0±0.1 µS and 2.0±0.2 µS for oocytes injected with mRNA encoding a DEG mutant αβγENaC). These results suggest that the αβγENaC-mediated Na permeability is increased in the oocytes expressing xShroom1 sense and antisense respectively (n=18).

REFERENCES: Jaffe et al., JCI 2010. Remal INsmth Fondation Transatlantic on Hypertension.
proteins has been proposed [4]. The aim of this study was to identify cleavage sites in human γENaC that are functionally important for channel activation by plasmin. Sequence comparison of human and mouse ENaC suggested a putative plasmin cleavage site in human γENaC (K189). To study its functional relevance we generated a γK189A mutant by site-directed mutagenesis and expressed wild-type and γK189A γENaC in Xenopus laevis oocytes. The γK189A mutation reduced but did not abolish the stimulatory effect of plasmin (10 μg/ml) on ENaC. In contrast, mutating a putative proline site (γRRK178AAA) had no apparent effect on the stimulatory response to plasmin. Interestingly, the combination of both mutations (γRRK178AAA γK189A) abolished the stimulatory effect of plasmin. We conclude that channel cleavage at a putative plasmin site and at a putative prostasin cleavage site is involved in mediating proteolytic activation of human ENaC by plasmin. This work was supported by the Interdisziplinäres Zentrum für Klinische Forschung (IZKF) and by the ELAN program of the University of Erlangen-Nürnberg. Reference: Rossier BC and Stutts MJ (2009). Annu Rev Physiol 71, 361-379.Svenningsen et al. (2009). J Am Soc Nephrol 20, 299-310. Pasero et al. (2008). J Biol Chem 283, 36586-91.Svenningsen et al. (2009). Am J Physiol Regul Integr Comp Physiol 297, R1733-41.

4.7 DOES INSULIN REGULATE ENaC?

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Physiology and Biophysics, Weil-Cornell Med. Coll., 1300 York Ave, New York, NY 10065. Insulin increases Na transport in vitro in amphibian epithelia via ENaC. Data on the hormone action on this transport in mammalian epithelia are scant and controversial. We tested the effect of insulin (2 nM, 30-60 min) on principal cells of isolated split-open rat CCD using whole cell current measurements. Insulin addition to the superfuse of the tubules dissected from control animals did not induce the appearance of amiloride-sensitive Na current, while in high-K fed animals insulin currents were present in the presence of amiloride (43±2 vs. control 19±2 pA/pC). However, the hormone enhanced Na-pump current (ouabain-sensitive) from 18±3 to 31±3 pA/C in control and from 74±9 to 126±11 pA/C in high-K fed animals. It also more than doubled ROMK (TPN-sensitive) K currents in control CCD from 32±44 to 69±82 pA/pC and from 23±0 to 111±23 pA/pC in high-K/Ko loads. Efforts to elucidate the mechanism contributing to an effect of Na on insulin excretion runs in vivo were unsuccessful. In summary, although the hormone does activate the Na pump and apical K channels, we find no evidence for up-regulation of ENaC by insulin in the mammalian CCD.

4.8 CLEAVAGE OF ENDOGENOUS γENaC AND ELEVATED ABUNDANCE OF γENaC IS ASSOCIATED WITH INCREASED Na+ TRANSPORT IN RESPONSE TO APICAL FLUID VOLUME EXPANSION IN HUMAN H441 AIRWAY EPITHELIAL CELLS

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Thoracic Sci., St. George’s Univ. of London, Cranmer Ter., Tooting, London, SW17 0RE, UK. We have correlated the functional response to apical fluid volume expansion with abundance and cleavage of endogenous α and γENaC proteins in the apical membrane of H441 airway epithelial cells cultured at air liquid interface (ALI). When placed in fluid-filled Ussing chambers, monolayers cultured at ALI rapidly elevated Isc (t1/2 = 2.3 mins). The increase in Isc was not mediated by an increase in Na currents, but rather a novel current (γENaC, 90 kDa) distributed to increased cleavage as protease inhibitors had no effect on the ratio of cleaved to non-cleaved (90 kDa) ENaC proteins. Instead, fluid expansion increased the abundance of 63-65kDa γENaC. The γK189A mutation reduced but did not abolish the stimulatory effect of plasmin (10 µg/ml) on the γENaC current. Interestingly, the combination of both mutations (γRRK178AAA RKRK178AAAA) had no apparent effect on the stimulatory response to plasmin. Interestingly, the combination of both mutations (γRRK178AAA RKRK178AAAA) abolished the stimulatory effect of plasmin. We conclude that channel cleavage at a putative plasmin site and at a putative prostasin cleavage site is involved in mediating proteolytic activation of human ENaC by plasmin.

5.0 DISEASE-CAUSING MUTATIONS AFFECT ENaC GATING

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Physiology, UTHSCSA, 7703 Floyd Dr, San Antonio, TX, 78229,1Integrative Biology and Pharmacology, UTHealth, 6515 Fannin St, Houston, TX 77030. The activity of the epithelial Na channel (ENaC) is a critical determinant of systemic Na levels and, thus, effective circulating volume and blood pressure. Gain- and loss-of-function mutations in ENaC affect renal Na handling to increase and decrease, respectively, blood pressure. The majority of disease-causing mutations are believed to influence channel expression level. Those that influence the biophysical properties of channels within the plasma membrane, though, are also capable of causing disease, and should be informative about structure-function relations within the channel protein. Here, we identify one gain-of-function Liddle’s and three loss-of-function PHA-I mutations in ENaC that lead to changes in single channel properties. The N530L Liddle’s mutation in the γ-subunit substitutes a conserved (in ENaC) Arg that is one position downstream of the Dog site. Channels containing γENaC harboring the N530S substitution have increased activity and open probability. Channels containing the PHA-I mutation, G375S in ρENaC, have decreased at physiological potentials. This critical Gly is an inappropriately conserved Hgly motif in the cytosolic NH2-terminal of the protein key to gating. Another PHA-I mutation, the KYS106-108→N substitution in γENaC, also markedly decreases current likely by decreasing open probability. The PHA-I mutation, S562P in γENaC, results in complete loss of function. S562 occupies a critical position in the selectivity filter of the channel pore possibly being involved in coordination of the conductive ion during permeation. Thus, this mutation is likely to affect either gating or permeation. Efforts to demonstrate an effect of insulin on Na excretion in vivo were unsuccessful. In summary, although the hormone does activate the Na pump and apical K channels, we find no evidence for up-regulation of ENaC by insulin in the mammalian CCD.
The epithelial sodium channel (ENaC) is a heterotrimer of homologous hENaC subunits. The C-terminus of ASIC1a subunits may be important for the subunit-cross-link depends on cysteines in the C-terminus. We confirmed by size exclusion chromatography that two Cys in the C-terminus of ASIC1a subunits were found primarily responsible for the inhibitory effect of Cu2+ on human ENaC based on experiments with mixed human and mouse ENaC subunits. The inhibition of hENaC by Cu2+ was pH dependent. Mutations of multiple His residues within extracellular domains significantly reduced the inhibition of human ENaC by Cu2+. We identified dH48 as a putative Cu2+ binding site at the subunit interface between thumb subdomain of hSNAP and palm subdomain of another counterclockwise subunit (viewed from above). The inhibition by Cu2+ was not dependent on an existence of N-termini self-inhibition, suggestive of a unique mechanism. We conclude that extracellular Cu2+ is a high affinity inhibitor of human ENaC and binds to sites within the extracellular domains including a subunit interface. (NIH R01 ES04701 and P30 DK079307).

4.16 EPITHELIAL SODIUM CHANNEL (ENaC) ACTIVITY AND GATING IS MODULATED BY PALMITOTYLY OF BOTH THE BETA AND GAMMA SUBUNITS

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Activity of ENaC is modulated by regulation of its membrane trafficking, binding of extra- cellular C-terminals such as Na+, and cytoplasmic interactions with inositol phospholipids. We recently reported that cytosolic ENaC activity is modulated by palmitoylation of cytotoxic cysteine residues (Mueller et al., 2010 J Biol Chem 285, 30453-62) Using fatty acid-exchange chemistry, we found that β and γ, but not α, are modified with palmitoyl. Analysis of mutant ENaC's revealed that two Cys in β were palmitoylated (Cys43 and Cys55), Xenopus oocytes expressing ENaC with mutant Cys43/557A had significantly reduced whole cell currents, enhanced Na+ selectivity, and reduced single channel P0. When compared with wild-type ENαC, while membrane trafficking, proteolytic processing and surface levels were unchanged. We now report that mutation of either of the two cysteine residues significantly reduced the inhibition of human ENaC by Cu2+. We identified dH48 as a putative Cu2+ binding site at the subunit interface between thumb subdomain of hSNAP and palm subdomain of another counterclockwise subunit (viewed from above). The inhibition by Cu2+ was not dependent on an existence of N-termini self-inhibition, suggestive of a unique mechanism. We conclude that extracellular Cu2+ is a high affinity inhibitor of human ENαC and binds to sites within the extracellular domains including a subunit interface. (NIH R01 ES04701 and P30 DK079307).

4.17 TMPRSS4 ACTIVATES THE EPITHELIAL SODIUM CHANNEL BY CLEAVING THE GAMMA SUBUNIT DISTAL TO THE FURIN CLEAVAGE SITE

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The epithelial sodium channel (ENαC) is activated by a unique mechanism whereby inhibitory peptides are released by proteolytic cleavage within the extracellular loops of two of its three subunits. While cleavage by furin within the biosynthetic pathway releases one inhibitory tract from the β subunit and moderately activates ENαC, full activation through release of a second inhibitory tract from the γ subunit occurs only by furin and at a distal site by a second protease such as proteinase, plasmin or elastase. We now report that co-expression of mouse TMPRSS4 with mouse ENαC in Xenopus oocytes was associated with a two- to three-fold increase in channel activity and production of a unique ~70 KDa C-terminal fragment of the γ subunit, similar to the ~70 KDa γ fragment we previously observed with proteinase-dependent cleavage activation. Channel activation by TMPRSS4 and production of the ~70 KDa γ fragment were partially blocked by mutation of the proteolytic cleavage site (RKRK186QQ). Complete inhibition of TMPRSS4 activation of ENαC γ subunit cleavage was observed when three basic residues between the furin and proteinase cleavage sites were mutated (K177Q, K175Q and R177Q) in addition to the proteolytic cleavage site (RKRK186QQ). We conclude that TMPRSS4 fully activates ENαC by cleaving basic residues within the tract γK173-K186 and thereby releasing a previously defined key inhibitor tract encompassing γR155F168, from the γ subunit. (NIH DK065161, DK079307, DK080574, and HL087932).

4.18 FUNCTIONAL CHARACTERIZATION OF THE PERMEATION PATHWAY OF ASIC1a

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Acid-sensing ion channels (ASICs) are cation selective channels that undergo activation and desensitization in response to extracellular acidification. The pore of ASIC1 is a hourglass-like shape with residues in the second transmembrane (TM2) helices forming the ion permeation pathway. To functionally define the localization of the closed gate of ASIC1a, we mutated residues in the TM2 helix to histidine and investigated the reactivity of these mutant channels toward mTRA in the closed and open states. Our studies indicate that ASIC1 channels are modified by MTRA in the closed state, A227C and G428C mutants are modified in both closed and open states, while L429C and G431C channels are modified only in the open state. Our result is consistent with the presence of an extracellular vestibule and gate, as suggested by the inhibition of activated toward extracellular acidification. The pore of ASIC1a contribute to cation selectivity.

4.19 MECHANISTIC BASIS FOR SPECIFIC ACTIVATION OF SGK1 BY mTOR

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The serum and glucocorticoid-induced kinase 1 (SGK1) plays an important role in hormone regulation of ENaC-dependent Na+ transport. We have previously reported that the mTOR complex 2 (mTORC2) activates ENαC by phosphorylating SGK1. Here we identify rSGN1 as the mTORC2 component that mediates interaction with SGK1, and demonstrate that this interaction is required for SGK1 phosphorylation and ENαC activation. We used the yeast two-hybrid system coupled with mammalian two-hybrid system to identify a mutant rSGN1 that does not interact with SGK1. Expression of this mutant does not restore SGK1 phosphorylation to wild-type levels in mSNOD3-deficient mouse embryonic fibroblasts. Furthermore, in kidney epithelial cells, the rSGN1 mutant has a dominant-negative effect on SGK1 phosphorylation and on SGK1-dependent mTORC2 activation. Interestingly, the role of rSGN1 in mTORC2 activation appears to be specific for SGK1, as rSGN1 is essential for phosphorylation of another mTORC2 substrate, Akt. In contrast, Akt is not affected by the point mutation that abrogates interaction with SGK1. These data support the conclusion that mTOR, which regulates a wide array of cellular processes, uses distinct strategies to phosphorylate its various substrates, and suggest a mechanism for specific regulation of ENaC-mediated Na+ transport without inadvertent effects on unrelated cellular processes.

4.20 IN VITRO AND IN VIVO INHIBITION OF THE MEMBRANE-BOUND SERINE PROTEASE/CAPI/Prox8 BY SERPINS

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Serine proteases are involved in the regulation of many biological processes like e.g., blood coagulation, wound healing, digestion, immune response and channel activation. This requires a tight regulation that may be achieved by specific serine protease inhibitors (serpins), and any alteration of this balance may lead to diseases. CaPI/Prox8 was the first of several membrane-bound serine protease found to activate ENαC. In our present study, we used the in vitro Xenopus oocyte expression system allowed the inhibitory effect of potential CaPI/Prox8 inhibitors on CaPI/Prox8-induced ENαC currents. Thereby, we identified a serpin that was able to block ENαC activation by CaPI/Prox8 in vitro. To verify its inhibitory effect in vivo, we generated mice transgenic for this serpin and crossed those with mice transgenic for CaPI/Prox8 in the skin that exhibit a scaly skin phenotype, an increased epidermal thickness and an excessive water loss through the skin. Strikingly, in double transgenic mice, this double defect phenotype was prevented strongly suggesting that the effects through CaPI/Prox8-over-expression is blocked by this inhibitor. In conclusion, we identified an inhibitor of CaPI/Prox8 that may well be implicated in the regulation of CaPI/Prox8 activity in various organs. This was supported by the National Science Foundation (Grant 20010-01325 to Edith Hammard).
4.21

THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) inhibits PROTEOLYTIC STIMULATION of ENaC

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The epithelial sodium channel (ENaC) and cystic fibrosis transmembrane conductance regulator (CFTR) are required for ion absorption and secretion at the apical membrane of epithelia in airways, intestines, and other tissues. Inactivation of ENaC by CFTR prevents excessive sodium absorption. In cystic fibrosis, the absence of functional CFTR results in hypertensive ENaC channels and dehydration of human airway surface. Limited proteolysis of ENaC’s extracellular domains regulates its open probability and we have demonstrated that CFTR markedly inhibits proteolysis and processing of ENaC. Furthermore, co-immunoprecipitation experiments revealed that several domains of CFTR, including the R-domain, interact with ENaC subunits. Proteolytic cleavage of both α- and γ-ENaC by channel activating protease 3 was drastically diminished in the presence of CFTR. To verify a role of CFTR in ENaC proteolysis in human airway epithelia, we analyzed the proteolytic state of ENaC, in normal and cystic fibrosis airway epithelial cultures that lack functional CFTR. Strikingly, in primary cystic fibrosis airway epithelial cells that were homozygous for AF508 CFTR the amount of proteolytically cleaved α- and γ-ENaC was significantly increased when compared to normal cultures. These observations suggest that CFTR protects ENaC from proteolytic processing by proteases in airway epithelia. Supported by the NIH [5R01 HL080561, 5P01 HL03422].

4.22

GENERATION OF MICE DEFICIENT FOR THE CHANNEL ACTIVATING PROTEASE 2/Trnpmp4

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Channel activating protease 2 (CAP2/Trnpmp4), a member of the family of membrane-bound serine proteases, has been identified as a channel-activating protease of ENaC-mediated sodium current. We have previously shown that ENaC activation in vitro requires and interacts CAP2 HDS catalytic triad. To study the effect of CAP2 in vivo, we generated mutant mice for CAP2/Trnpmp4, by using a replacement type vector that targets the histidine and aspartate of the catalytic triad. This targeting vector has been electroporated into mouse 129OEV ES cells, and correctly targeted clones were injected into blastocysts. Germine chimerae have been generated and CAP2lox+/- mice were born. Following breeding with Fp-mice and Nestin-CRE deleter mice, mice with CAP2lox and CAP2+/- alleles have been obtained. Currently, these mice are interbred to generate floxed CAP2lox and CAP2lox+/- mice. If they survive to adulthood, these mice will be analysed with respect to their capacity to induce ENaC-mediated sodium currents in kidney, colon and lung. This work is supported by the Swiss National Science Foundation (Grant 31000A-102125/1 to E. Hummler).

4.23

ROLE OF THE ESCRT PROTEIN Tsg101 IN THE TURNOVER OF THE EPITHELIAL Na+ CHANNEL IN THE KIDNEY

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Kidneys are the main regulator of salt homeostasis and blood pressure. In the distal region of the tubule active Na-transport is finely tuned. This transport is regulated by various hormonal pathways including aldosterone that regulates the reabsorption at the level of the ASDN, comprising the late DCT, the CNT and the CCD. In the ASDN, the amiloride-sensitive epithelial Na-channel (ENaC) plays a major role in Na-homeostasis, as evidenced by gain-of-function mutations leading to salt-sensitive hypertension. In this disease, regulation of ENaC is compromised due to mutations of ENaC that disrupt the ubiquitination of ENaC, leading to reduced endocytosis of the channel, and consequently to increased channel activity. These findings show that ROS play an important role in normal ENaC response, show predominately amiloride-sensitive electronic transport, retain expression of markers of principal cells (Calbindin, ROMK-1, SGLK, GLZ-M), and show markedly diminished expression of markers from other nephron segments (SGLT2, UTA-2). This method can be used to investigate differences between wild-type and transgenic mouse models. Funded by an ASN Career Development Award (VB) and NIH R03DK83613 (VB).

4.25

REGULATION OF Na+ HOMEOSTASIS BY THE DEUBIQUITYLATING ENZYME USP2 IN VIVO

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Na+ homeostasis is a key process for renal blood pressure regulation in mammals. Na+ reabsorption occur all along the renal tubule and about 15% is left under the hormonal control of Aldosterone in the Aldosterone-sensitive Distal Nephron (ASDN) through the Thiazide-sensitive Na+/Cl- Cotransporter (NCC) and the Amiloride-sensitive Epithelial Na+ Channel (ENaC). Both have been shown by our lab and collaborators to be regulated by ubiquitin mediated degradation via the SGT1-NEDD4-2 pathway. The deubiquitylating enzyme Usp2-45 was identified as an aldosterone-induced gene and in vitro studies showed that it enhances ENaC channel surface expression and activity. Moreover, USP2-45 interacts with the ubiquitin ligase NEDD4-2 and ENaC. Altogether, these data make USP2-45 a positive regulator of ENaC by counteracting its down-regulation by NEDD4-2. We here address the importance of Usp2 in Na+ homeostasis in vivo by taking advantage of a Usp2 knockout mouse model. We challenged these animals by their adaptation to dietary switch from Normal Sodium (0.17% Na+) to either Low Na+ (0.01% Na+) or High Sodium (3.2% Na+) Diets. We report here that the Usp2-KO mice adapt perfectly to Na+ dietary changes, display normal plasma aldosterone levels, comparable expression levels of NKS, NCC and NEDD4-2 and show no variation in blood pressure under LSD or HSD, suggesting that alternative regulatory mechanisms have taken place in these animals.

4.26

Withdrawn.

4.27

EPITHELIAL SODIUM CHANNEL (ENaC) DELTA SUBUNIT AND ITS FUNCTIONAL EXPRESSION IN HUMAN RESPIRATORY EPITHELIAL CELLS

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The α-, β- and γ-subunits of ENaC play a significant role in ion and fluid homeostasis in the lung, however, the function of the fourth homologous ENaC subunit, δ(ENaC) remains largely unknown. Here, we studied δENaC expression and its contribution to ion transport function in human respiratory epithelial cells in vitro. Expression of the δENaC subunit was investigated in human respiratory epithelial cell lines (A549, H441, Calu-3 and 16HBE135) and in primary cultures of alveolar epithelial type II cells on gene and protein levels. Pharmacological effects of δENaC modulators (Evans blue, capsaicin and icilin) were investigated in H441 and Calu-3 cell monolayers in Usning chamber studies. Messenger RNA transcripts encoding δENaC were detected in all investigated cell types. PCR data were confirmed by Western blot and confocal laser scanning microscopy. The reported modulators of δENaC function resulted in concentration-dependent blockade of Na+ current in H441 cell monolayers. Intriguingly, in Calu-3 cell monolayers only capsaicin showed an inhibitory effect, whereas Evans blue and icilin stimulated currents, which were likely Cl currents. Our data indicate that δENaC is expressed in all investigated cell types. The fact that reported modulators showed pronounced pharmacological effects suggests a transcellular role for δENaC in human respiratory epithelial cells. However, further studies need to determine the specificity of the observed pharmacological effects. ES is funded by an IRSCET postgraduate scholarship.

4.28

Rac1-MEDIATED NADPH OXIDASE PRODUCTION OF O2- REGULATES LUNG ENDOPLASMIC RETICULUM

My-Hsien1

Physiology, Emory Univ., 615 Michael St, Ste. 646, Whitehead Res. Bldg., Atlanta, GA, 30322. Our lab's goal is to achieve a better understanding of how epithelial sodium channel (ENaC) is regulated in the lung by reactive oxygen species (ROS). Using single channel patch clamp analysis, we initially found that sequestering O2- with tetramethylpyperidine-N-oxyl (TEMPO) immediately inhibits ENaC open probability (Po) from 0.10+ to 0.03 (n=15, P<0.005). Conversely, increasing ROS, with either a combination of xanthine oxidase and hypoxanthine reduced ENaC activity and reduced Po from 0.10 to 0.05 (n=15, P<0.005). We established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of NADPH oxidases that are involved in physiological and pathological processes in thelung.
4.29

**WNK4 INHIBITION OF ENaC IS INDEPENDENT FROM Ned4-2 MEDIATE ENaC UBIQUITINATION**

Ling Yu, Hui Cai, De Xuan Wang, Abdul Ali, Qiang Yue, Oter Akkahlili, Peter Snyder

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Epithelial sodium channels (ENaCs) in the distal nephron play key role in regulating Na+ reabsorption. A serine/threonine protein kinase, WNK4, expressed in this region inhibits Na+ and K+ reabsorption by reducing delivery of NCC to and enhancing ROMK channel retrieval from the apical membrane. WNK4 regulates ENaC in expression systems has also been described, but not how these results are functional. We investigated ENaC expression in A6 cells, both trans-epithelial current and single channel recordings show that WNK4 inhibits ENaC activity. Further analysis channel number in a patch showed that WNK4 reduces channel number but has no effect on channel open probability. Western blots of apical and basolateral ENaC provided evidence of ENaC as well as ENaC channel expression.

In the mechanism of WNK4-inhibition of ENaC, we found that WNK4 enhances ENaC endocytosis. We also examined whether WNK4 inhibits Ned4-2 mediated ENaC retrieval and found no additive effect in trans-epithelial current in WNK4 or Ned4-2 expressing cells is similar, but 20% of channel cancelation is stronger in Ned4-2 expressing cells. Lastly, we performed co-immuno precipitation experiment on ENaC and WNK4 and found that Liddle’s mutated ENaC is associated with WNK4. Our results demonstrate that WNK4 inhibits endogenously expressed ENaC by enhancing channel internalization, but this inhibition is independent of Ned4-2 mediated ENaC ubiquitination.

4.30

**MARCKS REGULATES ENaC BY REVERSIBLY SEQUESTERING PHOSPHATIDYLINOSITOL PHOSPHATES**


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Phosphatidylinositol phosphates (PIPs) stimulate epithelial sodium channels (ENaC) in the apical membrane of Na transporting epithelia. Neither PIPs nor ENaC are abundantly expressed in ENaCs, and it was shown that inhibition of ENaC by PIPs in a patch showed that ENaC reduces channel number but has no effect on channel open probability. Western blots of apical and basolateral ENaC provided evidence of ENaC as well as ENaC channel expression.

In the mechanism of WNK4-inhibition of ENaC, we found that WNK4 enhances ENaC endocytosis. We also examined whether WNK4 inhibits Ned4-2 mediated ENaC retrieval and found no additive effect in trans-epithelial current in WNK4 or Ned4-2 expressing cells is similar, but 20% of channel cancelation is stronger in Ned4-2 expressing cells. Lastly, we performed co-immuno precipitation experiment on ENaC and WNK4 and found that Liddle’s mutated ENaC is associated with WNK4. Our results demonstrate that WNK4 inhibits endogenously expressed ENaC by enhancing channel internalization, but this inhibition is independent of Ned4-2 mediated ENaC ubiquitination.

4.31

**CONTROLLING THE ENaC WITH LIGHT**

Matthias Schoenberger, Mike Althaus, Martin Fronius, Wolfgang Clara, Dirk Tannen

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The sodium selective voltage-gated epithelial sodium channel (ENaC) is widely expressed with low subunit content in both the proximal and basolateral epithelia. Confluent monolayer showed colocalization of MARCKS and PIP2 at the apical membrane and the translocation of MARCKS to the cytoplasm after an ionomycin-induced increase in intracellular calcium. Lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment. In contrast, the lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment. In contrast, the lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment. In contrast, the lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment. In contrast, the lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment. In contrast, the lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment. In contrast, the lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft fractions to high density lipid raft fractions after PMA treatment.
Olivier Staub1
UBIQUITYLATION-DEUBIQUITYLATION CYCLES IN THE CONTROL OF
REGULATION OF UBIQUITIN LIGASE ACTIVITY AND PHOSPHORYLATION
by SGK1
Vivek Bhalla2
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94304
Regulation of epithelial Na+ channel (ENaC) mediated transport in the distal nephron is a critical determinant of blood pressure in humans. Nedd4-2, a member of the Homology to the E6-associatced protein C (HEC) family of ubiquitin ligases, is a physically important inhibitor of ENaC. Site-specific phosphorylation has been uncovered as a general mechanism to regulate ubiquitin ligases to either increase or decrease their activity. Aldosterone stimulates ENaC through several pathways but importantly, via induction of sodium-glucose cotransporter 1 (SGLT1). These effects of Nedd4L loss are likely caused by another Nedd4L function, as reflected by increased ENaC protein levels, increased mRNA expression of the amiloride-sensitive Epithelial Na+ Channel (ENaC) in the airways, and absence of ENaC activation by extracellular proteases may be relevant in native tissue. How-
Sodium handling in the distal nephron is under control by RAAS with aldosterone increasing ENaC activity to drive sodium reabsorption and to compensate for volume depletion. However, the apparent aldosterone paradox exists which allows the kidney to effectively conserve Na⁺ without any apparent K⁺ wasting during changes in systemic salt intake. Here, we used patch clamp electrophysiology to investigate the role of aldosterone in ENaC regulation. Sodium intake is regulated by systemic salt intake independently of aldosterone. We found that spironolactone treatment, while decreasing ENaC membrane levels, did not prevent regulation of ENaC open probability by salt intake. This raises a possibility that other components of RAAS, such as Ang II, could not regulate ENaC activity by salt intake. Indeed, we found that Ang II in the range from 5 to 500 nM acutely and reversibly increases ENaC Po by 5 nM of Ang II having only a subtle effect. This stimulatory effect was abolished when AT1 receptors were inhibited with Losartan. We next probed if Ang II stimulates ENaC activity in the presence of saturated aldosterone levels. The stimulatory effect of Ang II on ENaC was preserved although blunted in DOCA-treated animals. Moreover, we found that Angiotensin-converting enzyme (ACE) plays an important role in cleaving locally produced kinins, such as Bradykinin (BK), to further stimulate ENaC activity. ACE inhibition augmented the inhibitory action of BK on ENaC and caused marked decreases in intracellular apical recycling compartment under basal conditions. Its phosphorylation by aldosterone/SGK1 induces 14-3-3 protein binding, suppresses its GAP activity, augments its expression level and permits ENaC trafficking to the apical membrane to augment Na absorption (Liang et al. Mol Biol Cell. 2010).

Corticosterone to aldosterone and underphysiologic conditions is expressed in the adrenal zona glomerulosa under tight control of circulating angiotensin II (Ang II) and serum potassium (K⁺). The mineralocorticoid receptor (MR) classically acts as an aldosterone-dependent transcription factor at hormone-response-elements (GRE) that it shares with its closest relative, the glucocorticoid receptor (GR). Besides contributing to water, electrolyte and blood pressure homeostasis, the MR can also elicit pathophysiological effects in the cardiovascular system, including inflammation and fibrosis. Because these effects are not mediated by the GR, additional signaling mechanisms for the MR have been postulated. Recent findings revealed interactions of MR with signaling molecules of different cellular compartments and a cross-talk between non-genomic and genomic MR effects. The additional MR-signaling components include plasmalemma receptors like the epidermal growth factor receptor as well as cytokonic components like calcineurin. As pathophysiologichal consequences of these interactions an enhanced secretion of extracellular matrix components and a reduction in glucose-6-phosphate dehydrogenase expression have been documented. In the molecular classical GRE-dependent signaling takes place but further hormone-responsive-elements have been described as well. Overall, these findings suggest compartment-specific signaling of the MR embedded in an intracellular signaling network (PLOS GR. 2015;14(1): e0059134). Groenewoud C, Wafik M, Rohs S, Seifert A, Mildenberger S, Ruhe S, Schrêw G, Geleé M. Mineralocorticoid receptor inhibits CREB signaling by calcineurin activation. FASEB J. 2010 Jun; 24(6):2010-9.

7.0: PLenary lecture
7.1 aberrant Rac1-MR pathway in salt-sensitive hypertension and metabolic syndrome
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Obese persons with metabolic syndrome often have associated with salt-sensitive hypertension. Proteinuria and cardiac dysfunction, and the serum aldosterone level in one-third of metabolic syndrome patients is clearly elevated. Salt loading aggravates the proteinuria and induces cardiac dysfunction. Dysfuction because of the MR can only be suppressed by suppression of serum aldosterone level. Indeed, aldosterone excess and a high-salt diet exert an unfavorable synergistic action on cardiovascular damage. In Dahl salt-sensitive (S) rats, however, despite significant suppression of serum aldosterone with a high-salt diet, salt loading paradoxically activated renal MR signaling, and hypertension and renal injury was markedly prevented by MR antagonists. Accordingly, we discovered an alternative pathway of MR activation in which Rac1, a small GTP-binding protein, activates MR (Nat Med 2008). Salt loading increased Rac1 activity in opposite directions: Rac1 upregulation in Dahl-S rats and downregulation in Dahl-subsalt (R) rats. Despite the suppression of serum aldosterone in Dahl-D rats showed increased MR signaling in the kidneys, suggesting the key role of Rac1 in modulating salt susceptibility. Moreover, several metabolic syndrome-related factors such as IL-6 induce Rac1 activation. Treatment of Rac1 inhibitors decreased blood pressure and attenuated renal damages in both salt-loaded Dahl-S rats and Dahl-D rats. All these findings suggest that the role of Rac1 in hypertension and renal injury may be mediated by MR antagonists or by the selective suppression of Rac1 activity. Abnormal activation of Rac1-MR pathway plays a key role in salt-sensitive hypertension and metabolic syndrome. Reference: Shihata S, Nogu M, Yoshida S, Kasuwari K, wamuran H, Tamaka H, Miyoshi J, Takiya Y, Fujita T. Modification of mineralocorticoid receptor function by Rac1 GTPase implication in proteinistic kidney disease. Nat Med 14: 1370-1376, 2008.
8.0 ALDOSTERONE AND ENaC

8.1 CHOLERA TOXIN ENHANCES SODIUM ABSORPTION ACROSS CULTURED HUMAN MAMMARY GLAND EPITHELIUM: NOVEL MECHANISMS OF REGULATION ENaC FUNCTION IN MAMMARY GLAND

Mike Althaus1, Patrick Krumm1, Teresa Giraldez2, Diego Alvarez de la Rosa3, Wolfgang Clauss4, Martin Frohn1


Cellular mechanisms to account for the low Na+ concentration in human milk are poorly defined. CFTR10A cells isolated from human mammary gland and grown in permeable supports, exhibited amiloride- and benzamil-sensitive ion transport (short circuit current, Isc) suggesting activity of the epithelial Na+ channel, ENaC. When cultured in the presence of cholera toxin (Ctx), MCF10A cells exhibited greater amiloride sensitive Isc at all time points tested (2h to 7d), an effect that was not reduced with Ctx washout for 1-24 hours. Similarly, the amiloride sensitive current in ENaC decreased by Ctx in the presence inhibitors for PKA (H-89), PTK (LY294002) and protein trafficking (brefeldin A). Additionally, the Ctx B subunit, alone, did not replicate these effects. RT-PCR and western blot analysis showed no significant increase in either the mRNA or protein expression for α, β, γ, γ-ENaC subunits. Likewise, Nedd4-2 abundance was not changed. Biotinylation analysis showed that Ctx increased β and γ-ENaC expression in the apical membrane. These results demonstrate that human mammary epithelium express ENaC, which can account for low milk Na+ concentration, and that Ctx enhances ENaC localization in the apical membrane. This mechanism of Ctx-enhanced ENaC function may provide clues regarding mechanisms in human mammary gland epithelium that regulate Na+ transport via ENaC, which will likely have implications for epithelia throughout the body. [NIH P20-RR017666 & KS Ag Exp Stn support].

8.2 THIO-L REACTIVE COMPOUNDS FROM GARLIC INHIBIT THE EPITHELIAL SODIUM CHANNEL (ENaC) – A POSSIBLE MECHA-NISM FOR LOWERING BLOOD PRESSURE?

Mike Althaus1, Patrick Krumm1, Teresa Giraldez2, Diego Alvarez de la Rosa3, Wolfgang Clauss4, Martin Frohn1


Garlic is well known as a natural remedy with beneficial effects against high blood pressure. However, the mechanisms by which garlic exerts its hypotensive effects are poorly understood. The regulation of blood pressure in the kidney is linked to transepithelial sodium reabsorption from primary urine and particularly the activity of epithelial sodium channels (ENaC). Therefore we questioned whether there might be any impact of compounds from garlic on ENaC. Human ENaC complexes, consisting of the α, β and γ subunits, were heterotypically expressed in Xenopus oocytes. Transmembrane currents (I0) were recorded by the two-electrode voltage-clamp technique. Garlic extract (GE) was made from 5 g of fresh garlic in 10 ml of oocyte ringer solution on ice for 1 h and further diluted with oocyte ringer. The application of GE dose-dependently decreased the I0 of ENaC expressing oocytes, peaking at 90 % inhibition with the highest concentration (1 %). The effect was not apparent on water-injected control oocytes. The decrease of I0 due to GE was not reversible and was fully sensitive to the ENaC inhibitor amiloride. In the presence of saturating concentrations of L-cysteine (20 mM), the effect of garlic was blocked. In sum, we found that data indicate that thiol-reactive compounds from GE irreversibly inhibit ENaC. Decreasing sodium reabsorption in the kidney epithelium might represent a mechanism for diuresis, natriuresis and consequently hypotension as attributed to the health benefits of garlic.

8.3 THE δ1 AND δ2 ENaC SUBUNIT FORMS MECHANOSENSITIVE CHANNELS WHICH CO-EXPRESSION WITH β AND γ SUBUNITS INCREASED SENSITIVITY TO ACTIN CYTOARCHITECTURE INJECTION IN RATS WITH CHRONIC HEART FAILURE

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Four different types of ENaC subunits have been identified (αδβγ) and it is known that channels consisting of the αδβγ and subsunits are activated by shear force (SF). However, little is known concerning the effect of SF on δ1δ2ENaC. Two isoforms of human δ-ENaC (δ1 and δ2) were heterotypically expressed together with β and γ in Xenopus oocytes. Transmembrane currents were recorded by the two-electrode voltage-clamp technique. SF was applied with a laminar fluid stream. Both δ1δ2 and δ2δ1 were activated by SF and there was no difference between the two isoforms. The SF effect was pH sensitive: Acidic pH values (pH 4-6) increased ENaC activity and decreased the SF effect. With alkaline pH values (pH 8-10) opposite results were observed: Alkaline pH inhibited ENaC activity, but increased the SF effect. Half-maximal SF activation was determined at pH 7.9 for δ1δ2 and at pH 8.3 for δ2δ1 comprising channels. These observations indicate that SF increases the open probability of δ1δ2ENaC. Preincubation with trypsin, known to activate ENaC by proteolytic cleavage, attenuated the SF effect of δ1δ2ENaC. In summary, δ1δ2ENaC are activated by SF. The extracellular loops, which are accessible for protons and proteases, are likely involved in mechanosensitive gating of ENαCs. In summary, the δ1δ2 and δ1δ2 ENαCs. Pre-
This work is to study the importance of ENaC-mediated Na+ absorption and K+ secretion by de-...

...have been developed using a tetracycline-inducible Cre system (Pax8-rTta/LC1) active along the...

...body weight (-10% and -18%, respectively), whereas control mice continued to gain weight...

...the mice expressing the LacZ-cre recombinase driven by the Aldo promoter. After one week of sodium-deficient diet the IR-

...Cre-recombinase driven by the AQP2-promoter. After one week of sodium-deficient diet the IR-

...these results suggest that insulin via IR increases ENaC activity affecting the channel open prob-

...inhibits ENaC mRNA expression and ENaC-mediated Na+ and fluid absorption in a Stat6-de-

...inhibiting ENaC activity which was observed in wild type mice. Immunostaining of ENaC subunits...
The mineralocorticoid receptor (MR), a member of the nuclear receptor superfamily of transcription regulators, mediates aldosterone-dependent changes in renal ion transport and electrolyte homeostasis. The functional and expression data suggest that two distinct forms of remodeling of the distal tubule were involved in the carbohydrate metabolism, suggesting an increased capacity for energy production.

8.21 POTASSIUM DIET, HYPERTENSION, AND REMODELING OF THE DISTAL NEPHRON

Hiroshi Ikenaga, Masayuki Miura, Laura Helene Schulz, Akihisa Senki, Helga Vitharana

The renal sodium transport system is modulated by the renin-angiotensin system (RAS), which is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.22 ALDOSTERONE INDEPENDENT ACTIVATIONS OF MR-Sgk1-enAc and tubular renin angiotensin systems in sodium sensitive hypertension in mice

Hisako Yamana, Tomoaki Ishigami, Shintaro Minegishi, Satoshi Umemura

The renin-angiotensin system (RAS) is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.18 MODULATION OF THE EPITHELIAL SODIUM CHANNEL (ENaC) ACTIVITY by NOREPINEPHRINE in CULTURED DUCT CELLS is PARTIALLY MEDIATED by α2-ADRENERGIC RECEPTORS

Morgi Mamsiy, Maro Berger, Christina Korfbruch

The epithelial sodium channel (ENaC) is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.17 IDENTIFICATION OF PROTEINS REGULATED BY 24-HOUR ALDOSTERONE TREATMENT in MURINE LATE DISTAL CONVOLUTED TUBULES and CONNECTING TUBULES

Sepeh Rezaei, Uli B Jensen, Robert A. Fenton, Helle A. Presterl, Jason D Hoffmann

The epithelial sodium channel (ENaC) is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.16 THE ROLE OF RENAL MINERALOCORTICOID VERSUS GLUCOCORTICOID RECEPTOR in OEDEMATOUS DISEASES

Cintia Cerimachi, Verónica Fonseca de Leon, Félix Frey, Edith Hammerle

The epithelial sodium channel (ENaC) is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.15 RECEPTOR IN OEDEMATOUS DISEASES

C. Korbmacher

The epithelial sodium channel (ENaC) is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.14 IDENTIFICATION OF PERMISSIVE INSERTION SITES FOR GENERATING FUNCTIONAL FLUORESCENT MINERALOCORTICOID-RECEPTORS

Diego Alvarez de la Rosa, Cristina Aguilar, Ivan Hernandez-Diaz, Fabian Lorenzo-Diaz, Teresa Giráldez

The epithelial sodium channel (ENaC) is involved in the regulation of blood pressure and blood volume. In this study, we investigated the effects of the renin-angiotensin system (RAS) on the expression of Na/K excretion transporters in the distal convoluted tubule (DCT) and the collecting duct (CD).

8.13 THE MINERALOCORTICOID RECEPTOR (MR)-DEPENDENT AND INDEPENDENT MECHANISMS will be analyzed in these diseases. Using floxed GR and MR mice, we generated kidney-specific MR and GR knockout mice. These studies indicate that renal sodium transporters are modulated by norepinephrine and is at least partially mediated by basolateral α1-adrenoceptors. This work was supported by the Bayerische Forschungsstiftung.
8.23 ANGIOTENSIN RECEPTOR ACTIVATION CONTRIBUTES TO IMPAIRED RENAL INSULIN RECEPTOR PHOSPHORYLATION, INCREASED γ ENaC CLEAVAGE AND VOLUME-DEPENDENT HYPERDIASTOLIC INSULIN RESISTANCE IN RENAL I/R. "Pulse" injury made 10/1 Rats
Shara Balaban1, Ruben Rodriguez1, Jose Vicencio1, Daisuke Nakano2, Akira Nishiyama1, M. Hamada1, S. Awad1, R. Dwyer1, M.O. Oztur1, Nat. Sci., UC Merced, 5300 N Lake Rd, Merced, CA 95343, Pharmacology, Kagawa Univ., 1730-86 Tanabe, Shinchi-cho, Kagawa, Japan, Physiology and Biophysics, State Univ. of New York at Buffalo, 242 Cary Hall, Buffalo, NY, 14214
Epithelial Nav Channels (ENaC) are regulated by the insulin signaling pathway in cultured cells. However, in vivo regulation of ENaC by insulin receptor (IR) activation and the interaction between insulin and aldosterone signaling on ENaC is not well understood. The hypothesis that IR activation promotes angiotensin receptor blocker (ARB)-induced decrease in aldosterone, we studied three groups of rats: 1) normotensive LETO (control strain), 2) untreated, hypertensive, insulin resistant OLETF and 3) OLETF + ARB (10 mg olmesartan/kg/d x 6 wk). The response of OLETF and OLETF + ARB to insulin (30 min; 3.8 ± 0.4 mmol/l vs 14.6 ± 2.4 mmol/l, which was effectively reduced by ARB (107 ± 2 mmol/l) and associated with a reciprocal 160% increase in UrNa+/V. Plasma aldosterone and insulin decreased 18% and increased 95%, respectively, in OLETF. Both plasma aldosterone and insulin decreased 29% and 42%, respectively with ARB. Phosphorylation of IR increased 43% with ARB suggesting that AT1 activation contributes to impaired insulin signaling in the kidney. Cleaved α- and γ-ENaC subunits positively correlated with decreased plasma aldosterone suggesting that α and γ cleavage (and presumably channel activation) is associated with aldosterone in rats in resistance. Increased α- and γ-ENaC cleavage correlated with decreased UrNa+/V indicating that, along with impaired IR activation, cleavage in vivo contributes to UrNa+/V regulation and SBP during insulin resistance.

8.24 PHYSIOLOGICAL MODULATION OF URINARY PROSTASIN IN NORMOTENSIVE VS. HYPERTENSIVE INDIVIDUALS. "Pulse" injury made 10/1 Rats
Annalisa Castagna1, Laura Chiecchi1, Francesca Pizzolo1, Kenichiro Kitamura2, Ricciarda SIVES INDIVIDUALS PHYSIOLOGICAL MODULATION OF URINARY PROSTASIN IN NORMOTENSIVE VS. HYPERTENSIVE INDIVIDUALS. "Pulse" injury made 10/1 Rats
Background: Prostasin is a TGFβ-related growth factor that, along with impaired IR activation, cleavage in vivo contributes to UrNa+/V regulation and SBP during insulin resistance. Cleaved α- and γ-ENaC subunits positively correlated with decreased plasma aldosterone suggesting that α and γ cleavage (and presumably channel activation) is associated with aldosterone in rats in resistance. Increased α- and γ-ENaC cleavage correlated with decreased UrNa+/V indicating that, along with impaired IR activation, cleavage in vivo contributes to UrNa+/V regulation and SBP during insulin resistance.

8.25 DIFFERENCES AMONG RENIN-ANGIOTENSIN SYSTEM BLOCKADE FOR HYPERTENSION. "Pulse" injury made 10/1 Rats
Abhijeet Todkar1, Marianna Di Chiara2, Dominique Loffing-Cueni2, Carla Bettin2, Natalia Multahanova1, Oliver Ewert1, Andrea Raffelli3, Muthukumar Gunasekaran1, Gian Luca Salvagno4, Patrizia Guarini1, Oliviero Maccio1
1Inst. of Physiology, Univ. of Zurich, Winterthurerstr. 190, Zurich, 8057, Switzerland, 2Inst. of Pharmacology, Univ. of Zurich, Winterthurerstr. 190, Zurich, 8057, Switzerland, 3Dept. of Pathol. and Lab. Med., Univ. of North Carolina, Chapel Hill, NC, 27599, USA, 4Dept. Life and Exp. Med., Univ. of Verona, Piazzale L.A. Scuro 10, Verona, 37134, Italy, 5Inst. of Physiology, Univ. of Zurich, Winterthurerstr. 190, Zurich, 8057, Switzerland, 6Dept. of Pathol. and Lab. Med., Univ. of North Carolina, Chapel Hill, NC, 27599, USA
Hypertension is an essential requirement for the disease. The Na channel-Nedd4L-proteasome system might be the important converging molecular system.

8.27 ALDOSTERONE DEFICIENCY DURING PREGNANCY IN MICE DOES NOT LEAD TO PREECLAMPSIA BUT RESULTS IN PLACENTA DYSFUNCTION, REDUCED LITTER SIZE, AND SMALLER PUPS
Abhijeet Todkar1, Marianna Di Chiara2, Dominique Loffing-Cueni2, Carla Bettin2, Natalia Multahanova1, Oliver Ewert1, Andrea Raffelli3, Muthukumar Gunasekaran1, Gian Luca Salvagno4, Patrizia Guarini1, Oliviero Maccio1
1Inst. of Physiology, Univ. of Zurich, Winterthurerstr. 190, Zurich, 8057, Switzerland, 2Inst. of Pharmacology, Univ. of Zurich, Winterthurerstr. 190, Zurich, 8057, Switzerland, 3Dept. of Pathol. and Lab. Med., Univ. of North Carolina, Chapel Hill, NC, 27599, USA, 4Dept. Life and Exp. Med., Univ. of Verona, Piazzale L.A. Scuro 10, Verona, 37134, Italy, 5Inst. of Physiology, Univ. of Zurich, Winterthurerstr. 190, Zurich, 8057, Switzerland, 6Dept. of Pathol. and Lab. Med., Univ. of North Carolina, Chapel Hill, NC, 27599, USA
Preeclampsia is a syndrome with severe hypertension and proteinuria during pregnancy. Clinical studies pointed to a compromised aldosterone-synthetic activity in some of the preeclamptic women. We used aldosterone-synthetic knockout (KO) mice to address the role of aldosterone deficiency. Two types of breeding were used: wild type (WT) males mating with KO females and KO males mating with WT females. KO female mice were neither hypertensive nor proteinuric throughout the entire gestation period but became hypertensive at the end of pregnancy. Litter sizes, bodyweights of pups and weights of placentas were significantly smaller in KO than in WT female mice. Moreover, KO female mice revealed many small haemorrhagic placentas indicating prenatal death of pups. Feeding a high salt diet (5% NaCl) during pregnancy improved litter size and body weights of the pups in KO female mice. Thus, aldosterone deficiency in pregnant mice does not lead to preeclampsia but may impair placental function and influence pup growth and survival. A high dietary NaCl intake improves to placental function. Supported by ZHIPS and SNF.

8.28 RECOVERY OF ENDOTHELIAL-DEPENDENT VASODILATION BY ACUTE INHIBITION OF EPITHELIAL SODIUM CHANNEL (ENaC) IN RATS MADE HYPERTENSIVE BY ANGIOTENSINE II (AII)
Mauricio Born1, Nicholas Soto2, Marta Padrón Belén3, Luisa Lotti4, Esteban Segundo5, Monica Marquez1, Xavier Figueroa1
1Physiology, P. Univ. Católica de Chile, Av B. O'Higgins 340, Santiago, 8563777, Chile. Inhibitors of the mineralocorticoid receptor improve the outcome in different models of hypertension, independently of its effects on renal function. Among possible targets, we hypothesized that in hypertension, ENaC presence may be enhanced in vascular endothelium; reducing endothelium-dependent vasodilation, probably by modifying the NaCa exchanger (NCX) function and NO signaling. To address this hypothesis, we induced hypertension in Sprague Dawley rats (98-200g) by infusing AII (120 mg/kg/day, osmotic pump). After 2 weeks, all treated rats presented additional arterial content of ENaC and NCX, as compared with sham operated controls. Endothelial function was assessed in phenylephrine-contracted thoracic aort rings. Acetylcholine (ACh)-induced relaxation was reduced in aortas of AII-treated rats, whereas isoproterenol-induced relaxation was similar to control. Basal ENaC blockade with amiloride (100 mM) fully restored relaxation to ACh, but did not modify endothelium-independent relaxation to isoprenaline. The effect of ENaC inhibitors on ACh-induced relaxation was abolished by NO blockade (l-NAME 100 µM). In contrast, in vessels of control rats, ENaC blockade did not modify the responses to ACh, or isoprenaline. We conclude that activation of the renin-angiotensin-aldosterone system increases ENaC endothelial activity, and ENaC blockade improves endothelium-dependent relaxation in AII hypertensive rats. Grants Fondecyt 1090757, Azollos AC717.

8.29 FUNCTIONAL ASSESSMENT OF THE EPITHELIAL SODIUM CHANNEL (ENaC) IN THE RAT HEART
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1Physiology, P. Univ. Católica de Chile, Av B. O'Higgins 340, Santiago, 8563777, Chile, 2ICBM, Univ. de Chile, Independencia 1027, Santiago, 65030499, Chile.
We studied the functional expression of the epithelial sodium channel (ENaC) in the rat heart, a potential novel way of sodium uptake in cardiac cells. Studies were performed in isolated adult rat and neonatal rat myocytes using ENaC blockers benzamil and aramidil. Peak Na currents (whole cell configuration) demonstrated the presence of currents sensitive to aramidil (0.2-1.0 µM). According to ENaC channels characteristics, inversion potential for aramidil sensitive current was +8.1 ± 2.1 mV (n=6). Furthermore, both aramidil and benzamil decreased calcium transients and diastolic calcium levels as measured with Fura-2 in isolated cardiac myocytes exposed to different concentrations of ENaC blocker benzamil (1µM), or 0.1µM aramidil significantly the inverse response to isoproterenol (0.1µM-IB). On the contrary, ENaC blockade with other inhibitor did not affect the preload-contraction.
response. These results are consistent with the expression of functional ENaC channels in visceral
nicotinic myocytes of rat heart that contribute to maintain intracellular sodium and calcium levels and normal contractility. Grants Fondsdey 1007575 Antillos Ac71.

8.30
ENaC POLYMORPHISMS ALTER ENaC CURRENT
Udoelina C. Ermisoupos and Peter M. Snyder
Univ. of Iowa.

The epithelial sodium channel ENaC, a heterotrimer of homologous α, β, and γ subunits, is
found at the apical surface of epithelia where it facilitates sodium absorption. It therefore plays a
pivotal role in the maintenance of salt and extracellular fluid balance. Gain-of-function mutations
in β- and γENaC cause Liddle’s syndrome, a severe form of salt-sensitive hypertension, making
ENaC a candidate gene for the pathogenesis of essential hypertension. Less is known about the
role that αENaC sequence variations play in the regulation of ENaC function. We hypothesized
that single nucleotide polymorphisms in αENaC not only modulate ENaC function and play a role in
the pathogenesis of hypertension. To test this hypothesis, we coexpressed human αENaC polymorphisms
in the SNP database and tested the effects of 12 polymorphisms on αENaC current. Compared to wild type ENaC, one polymorphism (A334T) decreased amiloride-sensitive current in oocytes. We speculate that this polymorphism may protect from salt sensitive hypertension.

9.0
REMEMBERING J. D. HORISBERGER AND D. J. BENOS

9.1
REMEMBERING JEAN-DANIEL HORISBERGER
Laurent Schmid1
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Jean-Daniel Horisberger died on April 1st 2009. He studied medicine in Lausanne, then he began a PhD thesis in the Department of Pharmacology under the mentorship of Jacques Diezi. He published several seminal papers on the in vivo action of angiotensin in rats and mice. His contribution was essential for the cloning of ENaC, then he discovered the activation of ENaC by proteases such as trypsin. In parallel he pursued an outstanding research on the structure and function of the Na+ATPase, providing a detailed understanding of the gating mechanism that controls the access of potassium or sodium from the extracellular solution to their binding sites. His science was characterized by the highest scientific and ethical standards, and remains an example for us and for our students. 2Horisberger, J., and Diezi, J. (1983) Ann J Physiol 245, F90-F99. 3Inaba, A. et al. (1998) J Physiol 111, 127-138. 4Li, C. et al. (2005) Proc Natl Acad Sci US A 102, 12706-12711.

10.0: ABSTRACT-BASED PRESENTATIONS

10.1
THREE COMMDFAMILY PROTEINS ARE LOCATED IN COLLECTING DUCT CELLS AND REGULATE ENaC
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1Physiology, Univ. of Otago, 270 Great King St, Dunedin, 9054, New Zealand, 2Sch. of Biological Sci., Univ. of Auckland, 3Symonds St, Auckland, 1142, New Zealand.
2Copper Metabolism Mjatr1 Domain-containing protein 1 (COMMD1) reduces amiloride-sensitive short circuit current (Isc,amil) in epithelial cells expressing ENaC. To investigate whether other COMMDFamily proteins also regulate ENaC we used co-immunoprecipitation and showed that COMMD1-interacts with ENaC. COMMD1, 3, 9 were widely expressed in kidney including the distal nephron. The protein expression was increased by the aldosterone (Prep) antagonist, spironolactone. In Xenopus oocytes, COMMD1 reduced ENaC currents. AMT inhibitors (amiloride) increased ENaC currents in oocytes. We measured amiloride-sensitive ENaC current using two-electrode voltage clamp (oocytes) recordings. We identified αENaC polymorphisms in the SNP database and tested the effects of 12 polymorphisms on αENaC current. Compared to wild type ENaC, one polymorphism (A334T) decreased amiloride-sensitive current in oocytes. We speculate that this polymorphism may protect from salt sensitive hypertension.

10.2
A MODEL OF PARTNERSHIP CO-OPTED BY TSG101 AND USP2-45 AND USP2-45 FOR THE NEGATIVE FEEDBACK LOOP OF THE MINERALOCORTICOID RECEPTOR PATHWAY
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After activation of a signalling pathway and cell response, mechanisms often take place to
downregulate prolonged response and avoid tissue damages (cancers, inflammations...). In the
context of aldosterone/MR signalling, it was shown that prolonged exposure to aldosterone
decreased MR expression via the proteasome, but the mechanisms of this feedback regulation
remain unknown. We were therefore interested in elucidating mechanisms involving MR signaling
termination. We first observed that MR was mono-ubiquitylated at the basal state and that
this modification was removed after aldosterone treatment. As for other nuclear receptor, we
found that MR interacted with Tsg101 at the basal state and this association was disrupted after
aldosterone treatment. We found that Tsg101 can stabilize MR, probably by maintaining its
mono-ubiquitylation. Because USP2-45 is an aldosterone induced deubiquitylating enzyme, we
wondered if USP2-45 was involved in the deubiquitylation of the receptor. We found that USP2-45
interacted with MR, removed its mono-ubiquitylation and decreased its expression via the
proteasome. Our data imply that Tsg101 is involved in MR degradation in the basal state and
protected by Tsg101. Aldosterone treatment stabilizes USP2-45 expression, which
interacts with MR and deubiquitylates the receptor. The removal of the mono-ubiquitin de-
stabilizes the MR/Tsg101 interaction and induces MR degradation via the proteasome by a so
far unknown mechanism. These results reveal the existence of a functional network involving
USP2-45 and Tsg101 into a negative feedback loop of the MR pathway that mediates the de-
gradation of MR in response to aldosterone.

10.3
ANALYSIS OF TWO SPONTANEOUS MOUSE AND RAT CAP1/PRSS8 MUTATIONS
Simona Fratessi1, Anna Koppar2, Samudra Muthu1, Justyna Iwaskiewicz1, Nicola Fowler-Joa3, Anne-Marie Merillat1, Chloé Sena1, Bernard C. Rossier1, Edith Hurnik4
1Dept. of Pharmacology & Toxicology, Univ. of Lausanne, Bugnon 27, Lausanne, 1005, Switzerland, 2Swiss Inst. of Bioinformatics, Univ. of Lausanne, Quartier Sorge, Bâtiment Génépode, Lausanne, 1015, Switzerland.
3Cap1/Prss8 is a membrane-bound serine protease able to activate the epithelial sodium channel ENaC. We revealed a critical role for this serine protease in skin homeostasis and recently identified CAP1/Prss8 variants that cause the 'frizzy' phenotype in a mouse mutant (P138C βγS568C). In contrast, Cys mutations of
αP138 or
βS568; and
γP138; and
βγS568 caused Liddle’s syndrome, a severe form of salt-sensitive hypertension, making
ENaC a candidate gene for the pathogenesis of essential hypertension. Less is known about the
role that αENaC sequence variations play in the regulation of ENaC function. We hypothesized
that single nucleotide polymorphisms in αENaC not only modulate ENaC function and play a role in
the pathogenesis of hypertension. To test this hypothesis, we coexpressed human αENaC polymorphisms
in the SNP database and tested the effects of 12 polymorphisms on αENaC current. Compared to wild type ENaC, one polymorphism (A334T) decreased amiloride-sensitive current in oocytes. We speculate that this polymorphism may protect from salt sensitive hypertension.

10.4
WISTROD DOMAIN OF THE EPITHELIAL SODIUM CHANNEL MODULATES CHANNEL GATING
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The epithelial sodium channel (ENaC) is a key component in the regulation of extracellular fluid volume and blood pressure, primarily by modulating Na+ absorption, mucus dehydration and stasis in cystic fibrosis (CF) lungs. Conversely, loss of ENaC absorption, mucus dehydration and stasis in cystic fibrosis (CF) lungs. Conversely, loss of ENaC function results in increased MR expression via the proteasome by a so
far unknown mechanism. These results reveal the existence of a functional network involving
USP2-45 and Tsg101 into a negative feedback loop of the MR pathway that mediates the de-
gradation of MR in response to aldosterone.
The active site of SPLUNC1 was determined to be within an 18 amino acid region located near the N-terminus. We synthesized a peptide corresponding to this region, S18, and its inhibitory mechanism is being determined. Through binding studies with individual ENaC subunits we determined that S18 interacts with only the β-ENaC subunit. This interaction is being further explored using β-ENaC truncants to determine the specific site of interaction between S18 and ENaC. Addition of this peptide to CF cultures blocked Na+ hyperabsorption for 72 hrs, even in the presence of neutrophil elastase, suggesting that this peptide may have therapeutic potential. Understanding how S18 inhibits ENaC activation is key to the development of therapeutic peptides for the treatment of CF patients.

10.6 STEROID REGULATION OF THE ENAC RECYCLING PATHWAY: A PROTEOMIC ANALYSIS
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ENaC recycling to the apical membrane in kidney cortical collecting duct cells through sequential endosomal compartments. We have demonstrated ENaC flow through early endosomal antigen (EEA1) and Rab11 positive compartments. The size of the ENaC recycling compartment is regulated by ENaC expression which diminishes in the absence of steroids (SD) and is restored by steroid replacement (SR). We propose that steroids regulate specific components of the recycling pathway in addition to ENaC and have used an unbiased proteomic approach to examine the relative proteome of EEA1 and Rab11 endosomes under conditions of SD and SR. Using five different approaches (ITAC and DIGE), the goals of each compartment were identified. Several proteins were recognized as up-regulated in SR cells and have been confirmed by Western Blot. One major protein induced is Annexin 2 (Anx2). Anx2 has been implicated in both exocytic and endocytic pathways and has been described to form a functional complex with both EEA1 and Rab11 in vitro. Anx2 localization to Rab11 endosomes appears abundant in these endosomes with the addition of aldostosterone. Knockdown of Anx2 results in a decrease of basal and forskolin induced ENaC activity. These results suggest that Anx2 localizes to recycling endosomes upon addition of aldosterone and facilitates the recycling of ENaC. (Supported by NIH).

10.7 POSSIBLE VASCULAR ENaC INHIBITION BY AMILORIDE IN YOUNG OVERWEIGHT PREHYPERTENSIVES
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1IM, GHSU, 15th St, Augusta, GA, 30912, 2Peds, GHSU, 15th St, Augusta, GA, 30912, 3IM, Indiana Univ., Barnhill Dr. Indianapolis, IN, 46202.

Background: Recent data demonstrate the presence of the epithelial sodium channel (ENaC) in endothelial and smooth muscle cells. However, the function of vascular ENaC remains unknown in humans. Therefore, we aimed to determine the effects of ENaC inhibition on vascular function and structure in prehypertensives. Methods: Young black and white overweight prehypertensives (n=15, 19-35 yrs; body mass index: 29.5±1.6 kg/m²; 46% female; 46% blacks) were enrolled and received amiloride 10mg/day. Blood pressure (BP), brachial artery flow-mediated dilation (FMD), and pulse wave velocity (PWV) were measured at baseline, 2 and 4 weeks. Results: After 4 weeks of amiloride treatment, but not 2 weeks, a significant reduction was observed in both systolic (40.1±1.6 mmHg) and diastolic BP (37.1±2.2 mmHg). However, compared to baseline (51.1±11.0%), FMD was increased as early as at 2 weeks (6.4±1.2%, p=0.045), and further increased at 4 weeks (8.2±1.3%, p=0.024). On the other hand, compared to baseline (84.0±7.6 mmHg), carotid-radial PWV was decreased as early as at 2 weeks (78.0±6.0 mmHg, p=0.054), and further decreased at 4 weeks (76.0±5.0 mmHg, p=0.012). These tests were adjusted for gender, race, and systolic/diastolic BP. Conclusion: Amiloride appears to improve vascular function and structure in prehypertensives. The effects may be independent of blood pressure reduction, suggesting the inhibition of vascular ENaC. This study was funded by the Medical School of Georgia, GHSU.

10.8 POLYCLONAL ANTIBODIES AGAINST EPITHELIAL SODIUM CHANNEL (ENaC) SUBUNITS REVEAL DISTINCT PATTERNS OF ENaC EXPRESSION AND SUBCELLULAR LOCALIZATION IN HUMAN TISSUES
Yehoshua Enuka1, Israel Hanukoglu1, Oded Edelheit1, Hananya Vaknine2, Aaron Hanukoglu3
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We recently reported that reactive oxygen species (ROS) such as superoxides positively regulate uniformly expressed in epithelial cells, in others ENaC is expressed in subpopulations of epithelial cells. The high resolution of imaging also permitted us to distinguish association of ENaC with significant subcellular structures.

10.9 ENERGETIC AND STRUCTURAL BASIS FOR ACTIVATION OF ENaC BY CAP-3
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Limited proteolysis by endopeptidase is a ubiquitous phenomenon underlying regulation and activation of many enzymes and other proteins synthesized as inactive precursors. Serine proteases are among the largest families of endopeptidases involved in many cellular processes like wound healing and immune response. Heteromeric αγδ-epithelial sodium channels (ENaC) implicated in diseases like cystic fibrosis and Liddle’s syndrome are activated by type II transmembrane serine proteases (TSSP) and form-like converts. Despite identification of protease cleavage sites, the basis for enhanced susceptibility of αγδ-ENaC to proteases remains elusive. Here, we elucidate the energetic and structural bases for activation of ENaC by the TSSP CAP3. We find a region near the γ-ENaC furin site is previously unidentified as a critical cleavage site for CAP3-mediated stimulation. CAP3 also mediates cleavage of ENaC at basic residues downstream of the furin site. Our results indicate that surface proteases alone are sufficient to fully activate ENaC, and explain how ENaC in presence of surface-active proteases appears refractory to soluble proteases. Our results support a model where proteases prime ENaC for activation by cleaving at the furin site, and downstream cleavage is accomplished by membrane surface proteases or extracellular soluble proteases. In future, we will examine whether the accessibility of the CAP3 cleavage sites in ENaC is modulated by intracellular proteases, extracellular conditions and proximity to CFTR.
under normal or pathological conditions remain unclear. It is, however, clear that ROS can regulate the activity of an E3 ubiquitin ligase, E3-SCF, which consists of the skip, culin, and the F-box proteins. The activity of this E3-ligase complex is in turn regulated by another posttranslational event which requires neddylation of the culin subunit. We have shown that ROS directly inhibits neddylation of the culin subunit, and thereby inhibits E3-SCF enzymatic activity. If ENaC interacts with this E3-SCF ligase complex, then this may well be a novel mechanism by which ROS can regulate ubiquitin-mediated degradation of ENaC. Using standard immunoprecipitation techniques, we show for the first time that ENaC interacts with proteins associated with the E3-SCF ubiquitin ligase complex in type 1 and type 2 cells. Single channel patch clamp analysis confirm that MLN4924, a specific inhibitor of the neddylation pathway, significantly increased ENaC activity; ENaC NP values increased from 0.07 ± 0.03 to 0.80 ± 0.16 in type 2 cells treated with 10-100 nM MLN4924 for 60 minutes (P<0.05; n=8). Likewise, our preliminary results in type 1 cells show that Na current increases by -60 ± 60 min MLN4924 treatment (n=4). These increases in sodium channel activity indeed correlated with significant increases in surface expression of ENaC following MLN4924 treatment in type 1 and type 2 cells. Together, these findings provide mechanistic insight into how aldosterone could regulate ENaC activity in response to oxidative stress.

12.0: CONGESTIVE HEART FAILURE: THE INTERTWINED ROLES OF WATER AND SALT

12.1 POTENTIAL FUTURE ROLE OF MINERALOCORTICOID RECEPTOR BLOCKADE (MRB) IN PATIENTS WITH HEART FAILURE (HF)

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The kidney of vertebrates plays a major role in the homeostasis of the extracellular fluid. Despite large changes in salt intake, the kidney is able to maintain the extracellular osmolality and volume within very narrow margins. During the evolution of vertebrates, aldosterone played a critical physiological role about 300 million years ago with the emergence of amphibia, the first "protists" to multicellular metazoan, we provide here a novel view of how mammalian aldosteronism emerged. We propose that Na,K-ATPase emergence, together with ENaC/Degenerin, is linked to the development of multicellularity in the Metazoan kingdom. The establishment of volume hyperabsorption. Surprisingly, despite the efficacy of this peptide, full length SPLUNC1 fails to regulate ENaC in CF airways and knockdown of SPLUNC1 was without effect. Thus, we speculate that regions of SPLUNC1 adjacent to the inhibitory site prevent SPLUNC1-ENaC interaction in CF airways, and that the interaction is abolished when the inhibitory site is administred to CF airways alone. Thus, we propose that SPLUNC1 is an important regulator of ENaC which fails to function in CF airways. In addition, our results make it clear that the effectiveness of slowing ENaC proteolytic stimulation depends on the presence and function of CFTR. These results suggest that the use of SPLUNC1 inhibitors in CF may have therapeutic benefits for the treatment of CF lung disease. Funded by the NIH and the CFF.

14.4 EXPRESSION AND FUNCTION OF A CROSS CLADE ASIC/ENaC CHANNEL IN GLOBLASTOMA

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High grade glioma cells express an amiloride-sensitive cation conductance that is not present in normal astrocytes or in lower grade gliomas. This current is also inhibited by Pertussis toxin (PTxI), suggesting that at least one component of the channel is ASIC. Knockdown of ASIC1, aHNaC or gHNaC abolished the glioma cation current. However, knocking down ENaC was without effect. Furthermore, ASIC1 co-innumpregnated with both a- and gHNaC, but not with dHNaC, consistent with an ASIC1aNaHNaCh channel. Two of the hallmarks of a glioblastoma are its ability to grow to very large size and to migrate long distances, establishing multiple foci. We have found that benzamil and PTxI slow down both proliferation and migration of glioma cells. Cell cycle analysis showed that these inhibitors arrested cells at the G0/G1 checkpoint and caused an increase in expression of the cyclin kinase inhibitors p21Cip1 and p27Kip1. Benzamil and PTxI downregulated expression of pERK1/2, a regulator essential for cell migration. Similar results were obtained when external Na+ was replaced by NMDG+ or when ASIC1 was knocked down. These results suggest that expression of this conductance is important for migration and proliferation. We have now developed a peptide, supported by NHLBI 086996 and 51971).

14.5 ROLE OF ENaC IN THE REGULATION OF AIRWAY SURFACE LIQUID VOLUME AND PATHOGENESIS OF LUNG DISEASE

Marcus Mall1

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ENaC mediates myogenic constriction and plays an important role in control of RBF. ENaC mRN mice have increased renal expression of inflammatory and remodeling markers, such as macrophage infiltration, IL-1β, IL-6, TNFα, collagen III and TGFβ, suggesting loss of myogenic control is associated with mild renal injury. Moreover, ENaC mRN mice increased mean arterial blood pressure (MAP, 130 ± 3 vs. 105 ± 2 mm Hg, P<0.01), as measured using radio telemetry. Our findings suggest ENaC is an important mediator of renal myogenic constriction in vivo and loss of the myogenic mechanisms is associated with mild signs of renal injury and increased MAP. (Work supported by NHLBI 086996 and 51971).

14.3 REGULATION OF ENaC AND AIRWAY SURFACE LIQUID VOLUME BY SPLUNC1

Robert Tarran1, Carey Hobbs1, Sorpion Benachaj1, Jack Stut1

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Human bronchial epithelial cultures (HBEBC) utilize ENaC to regulate Na+ absorption and maintain airway liquid homeostasis (ASL). In contrast, cystic fibrosis (CF) epithelia fail to regulate Na+ absorption leading to ASL depletion. We identified SPLUNC1 as a potential sodium regulator of ENaC in CF, reduces its proteolytic activation, and decreases ENaC surface expression. Furthermore, NL HBECs with knocked down SPLUNC1 lost the ability to restrict Na+ absorption, suggesting that SPLUNC1 is an important soluble regulator of ENaC. We have recently identified the inhibitory region of SPLUNC1, and a peptide derived from this region robustly inhibits CF ASL volume hyperabsorption. Surprisingly, despite the efficacy of this peptide, full length SPLUNC1 fails to regulate ENaC in CF airways and knockdown of SPLUNC1 was without effect. Thus, we speculate that regions of SPLUNC1 adjacent to the inhibitory site prevent SPLUNC1-ENaC interaction in CF airways, and that the interaction is abolished when the inhibitory site is administered to CF airways alone. Thus, we propose that SPLUNC1 is an important regulator of ENaC which fails to function in CF airways. In addition, our results make it clear that the effectiveness of slowing ENaC proteolytic stimulation depends on the presence and function of CFTR. These results suggest that the use of SPLUNC1 inhibitors in CF may have therapeutic benefits for the treatment of CF lung disease. Funded by the NIH and the CFF.

14.2 THE ROLE OF BENAC IN RENAL VASCULAR FUNCTION

Heather Drummond1, Ying Ge1, Runsheng Liu1, Samira Grifoni1, David Stec1

14.3 PATTERN RECOGNITION ROLE OF THE ENRICH FAMILY IN THE REGULATION OF AIRWAY SURFACE LIQUID VOLUME AND PATHOGENESIS OF LUNG DISEASE

Marcus Mall1

Div. of Ped. Pulmonary and CF Clin., Dept. of Pediatrics III, Univ. of Heidelberg, Im Neuenheimer Feld 430, Heidelberg, 69120, Germany.

ENaC mediates myogenic constriction and plays an important role in control of RBF. ENaC mRN mice have increased renal expression of inflammatory and remodeling markers, such as macrophage infiltration, IL-1β, IL-6, TNFα, collagen III and TGFβ, suggesting loss of myogenic control is associated with mild renal injury. Moreover, ENaC mRN mice increased mean arterial blood pressure (MAP, 130 ± 3 vs. 105 ± 2 mm Hg, P<0.01), as measured using radio telemetry. Our findings suggest ENaC is an important mediator of renal myogenic constriction in vivo and loss of the myogenic mechanisms is associated with mild signs of renal injury and increased MAP. (Work supported by NHLBI 086996 and 51971).
14.6. PLASMIN, ENAC AND NEPHROTIC SYNDROME

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We found that nephrotic urine from the rat PAN-model and from patients activate the epithelial sodium channel (ENaC) in model systems – mainly mouse collecting duct M1 cells. The activation depended on serine protease activity, which was identified by MALDI-TOF mass spectrometry to be plasmin. Consistent with this, purified plasmin activated ENaC currents and inhibitors of plasmin abolished urinary protease activity and the ability of nephrotic urine to acti-

vate ENaC. The activation by plasmin involved cleavage of an inhibitory peptide from the ENaC, as shown using extracellular loop 2 of the M1-ENaC. Plasmin binding to the surface of the M1-ENaC and its binding depended on a GPI-anchored protein, which was identified by plasmin biotin-label transfer to be prostatin. Removal of GPI-anchored proteins inhibited stimulation of ENaC only at low plasmin concentrations (1-4 µg/ml). Consistent with this, knockdown of prostatin blocked plasmin-induced ENaC activity that was 100 fold greater than the whole-cell patch clamp technique. In nephrotic urine plasmin was likely to be converted from filtered plasminogen by tubular urokinase-type plasminogen activator (uPA). Thus, the uPA-inhibitor amikacin blocked production of plasmin in nephrotic rat urine in vivo. Conclusion, a defective glomerular filtration barrier allows passage of pro-inflammatory exogenous enzymes circulating levels of plasma aldosterone and renin and blood pressure. In contrast, the amino acid changing SNP in exon 2 MR I180V (rs5522) affects the cortisol-dependent tran-

membrane expression of the MR in vitro; importantly, this SNP influences the cortisol-dependent tran-

expression profile in untreated KO mice compared to WT. Loss of MR from myocytes prevented the DOC/salt-increased increase in NOX2, CCR5 and PAR2 mRNA. At 8 weeks, loss of myocyte MR prevented the DOC/salt-induced increase in infiltrating macrophages and T-cells, collagen deposition and systolic blood pressure. KO mice also showed a normal pressure response. Consistent profile in untreated KO mice compared to WT. Loss of MR from myocytes prevented the DOC/salt-induced increase in CR5, CD14 and CD81 mRNA was prevented in KO mice, whereas the DOC/salt-

inhibited increases in fibronectin, CTGF, COL-3, MCP-1 and DCN were unaffected. These findings suggest a direct role for myocyte MR signaling in DOC/salt-induced tissue remodelling, the secondary inflammatory phase. Reference: Sechi LA et al. Long-term renal outcomes in patients with primary aldosteronism. JAMA 2006;295:2638-2645. Catena C et al. Cardiovascu-


15.4. CARDIOMYOCYTE MR SIGNALING IS ESSENTIAL FOR DOCSALT-MEDIATED CARDIAC FIBROSIS AND BLOOD PRESSURE REGULATION

Amanda Rickard1, James Monroy1, Greg Castron1, Monas Young1


Inappropriate mineralocorticoid receptor (MR) signaling in the cardiovascular system is associated with inflammation and remodeling. However the mechanisms responsible and the contribution of the different cells of the myocardium remain unknown. We investigated the role of cardiac myocyte MR in an in vivo model of DOCSalt-induced pathology. Myocytes from wild-type (WT) or myocyte-specific MR null mice (KO) were examined after 8 days or 8 weeks of DOCSalt administration and compared to control mice (WT). At 8 days loss of MR signaling in myocytes did not alter DOCSalt-induced cardiomyocyte dysfunction, whereas limited early collagen deposition. Microarray revealed a novel gene expression profile in untreated KO mice compared to WT. Loss of MR from myocytes prevented the DOCSalt-induced increase in NOX2, CCR5 and PAR2 mRNA. At 8 weeks, loss of myocyte MR prevented the DOCSalt-induced increase in infiltrating macrophages and T-cells, collagen deposition and systolic blood pressure. KO mice also showed a normal pressure response. Consistent profile in untreated KO mice compared to WT. Loss of MR from myocytes prevented the DOCSalt-induced increase in CR5, CD14 and CD81 mRNA was prevented in KO mice, whereas the DOCSalt-

induced increases in fibronectin, CTGF, COL-3, MCP-1 and DCN were unaffected. These findings suggest a direct role for myocyte MR signaling in DOCSalt-induced tissue remodelling, the secondary inflammatory phase. Reference: Sechi LA et al. Long-term renal outcomes in patients with primary aldosteronism after treatment. Arch Intern Med 2008;168:80-85.
SCOPE OF JOURNAL

The American Journal of Physiology-Renal Physiology publishes original manuscripts on a broad range of subjects relating to the kidney, urinary tract, and their respective cells and vasculature, as well as to the control of body fluid volume and composition. Studies may involve human or animal models, individual cell types, and isolated membrane systems. Authors are encouraged to submit reports on research using a wide range of approaches to the study of function in these systems, such as biochemistry, immunology, genetics, mathematical modeling, molecular biology, and physiological methodologies. Papers on the pathophysiological basis of disease processes of the kidney, urinary tract, and regulation of body fluids are also encouraged.

Authors are required to submit papers online at www.apscentral.org.

EDITOR’S SELECTED ARTICLES

- **A novel lipid natriuretic factor in the renal medulla: sphingosine-1-phosphate**
  Qing Zhu, Min Xia, Zhengchao Wang, Pin-Lan Li, and Ningjun Li
  First published 6 April 2011; doi:10.1152/ajprenal.00014.2011

- **Assessing vesicoureteral reflux in live inbred mice via ultrasound with a microbubble contrast agent**
  Jose Paredes, Sunder Sims-Lucas, Hang Wang, Weinling Lu, Brian Coley, George K. Gittes, and Carlton M. Bates
  First published 16 February 2011; doi:10.1152/ajprenal.00720.2010

- **Acid retention accompanies reduced GFR in humans and increases plasma levels of endothelin and aldosterone**
  Donald E. Wesson, Jan Simoni, Kristine Broglio, and Simon Sheather
  First published 26 January 2011; doi:10.1152/ajprenal.00587.2010

- **Renal cortical albumin gene induction and urinary albumin excretion in response to acute kidney injury**
  Lorraine B. Ware, Ali C. M. Johnson, and Richard A. Zager
  First published 8 December 2010; doi:10.1152/ajprenal.00654.2010

- **Major role for ACE-independent intrarenal ANG II formation in type II diabetes**
  Sungmi Park, Benjamin J. Bivona, Hiroyuki Kobori, Dale M. Seth, Mark C. Chappell, Eric Lazartigues, and Lisa M. Harrison-Bernard
  First published 21 October 2009; doi:10.1152/ajprenal.00519.2009

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