Renal and extrarenal regulation of potassium

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The ISN Forefronts in Nephrology Symposium took place 8–11 September 2005 in Kartause Ittingen, Switzerland. It was dedicated to the memory of Robert W. Berliner, who died at age 86 on 5 February 2002. Dr Berliner contributed in a major way to our understanding of potassium transport in the kidney. Starting in the late 1940s, without knowledge of how potassium was transported across specific nephron segments and depending only on renal clearance methods, he and his able associates provided a still-valid blueprint of the basic transport properties of potassium handling by the kidney. They firmly established that potassium was simultaneously reabsorbed and secreted along the nephron; that variations in secretion in the distal nephron segments play a major role in regulating potassium excretion; and that such secretion is modulated by sodium, acid–base factors, hormones, and diuretics. These conclusions were presented in a memorable Harvey Lecture some forty years ago, and they have remained valid ever since. The concepts have also provided the foundation and stimulation for later work on single nephrons, tubule cells, and transport proteins involved in potassium transport.

Kidney International (2007) **72,** 397–410; doi:10.1038/sj.ki.5002288; published online 13 June 2007

KEYWORDS: potassium channels

Dr. Berliner, a native of New York, attended Yale University and received his medical education at Columbia University. After house-staff training in New York, he became assistant professor of medicine at Columbia in 1947 before moving to the National Institutes of Health in 1950. There he founded and led for many years the Laboratory of Kidney and Electrolyte Metabolism, one of the world's outstanding laboratories in kidney research. He was a highly critical and creative investigator who played a pivotal role in advancing kidney research, especially elucidating the renal mechanisms controlling salt, acid–base, and fluid balance. As a dominating pioneer in renal research, he was successful in attracting many excellent young investigators to his laboratory at the National Institutes of Health, many of whom became leaders in their field.

After occupying several key administrative positions at the National Institutes of Health, Dr. Berliner became dean of the Yale University School of Medicine, yet he never lost his interest in renal physiology. He attended scientific seminars, was always available for helpful advice, and never tired of editing and greatly improving the many manuscripts given to him for review. Dr. Berliner was an inspiring scientist of great integrity and modesty, and we were all better for knowing him (Figure 1).

This Forefronts Symposium on Renal and Extrarenal Regulation of Potassium dealt with several major aspects of potassium metabolism. It focused on the molecular structure, function, and regulation of potassium transporters and potassium channels, and surveyed relevant aspects of gastric and colonic potassium transport. Also considered were genetic transport lesions and mechanisms underlying disturbances of renal potassium transport. The meeting's first presentation was a plenary lecture delivered by Michel Lazdunski (Centre National de la Recherche Scientific, France), 'Sensing with K Channels.' A brief survey highlighting the main factors responsible for the unique internal distribution and for the regulation of potassium excretion by the kidney preceded the symposium presentations.

Gerhard Giebisch, Reto Krapf, and Carsten Wagner
Organizers

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Received 8 November 2006; revised 7 March 2007; accepted 8 March 2007; published online 13 June 2007

Figure 1 | (a) Bob Berliner. (**b**) Bob Berliner and his wife Lea celebrating the end of his deanship at Yale Medical School.

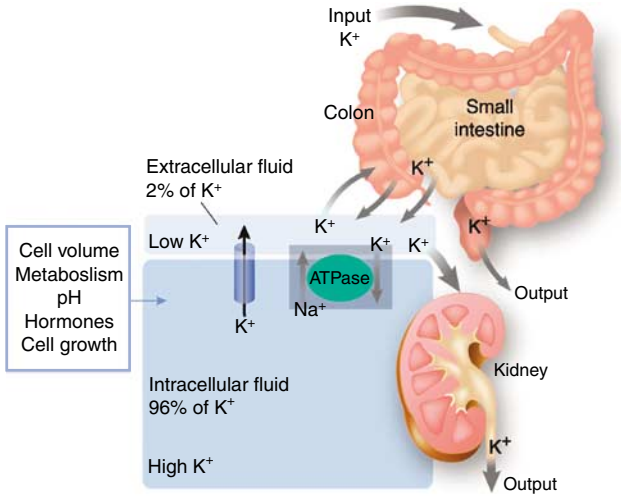

Figure 2 | Distribution of potassium (K) in the body. Most of K is located in body cells. Note entry of K into the small intestine, where it is extensively reabsorbed. K may be secreted in the colon. K's exit from the body is mediated both by the kidney and, to a lesser extent, by the colon. The distribution of K between extra- and intracellular fluid is dependent and regulated by a pump-leak mechanism involving both Na-K-ATPase and membrane K channels. Several factors modify cell K (see box).

INTRODUCTION

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Renal and extrarenal components of potassium homeostasis

Potassium (K) is the main intracellular cation, and proper distribution between extra- and intracellular fluid compartments as well as its effective excretion must be tightly controlled to maintain a narrow range of K concentrations in the blood.[1–3] Figure 2 provides an overview of the main routes of K intake and excretion. Intestinal K reabsorption from the small intestine into the extracellular fluid depends on transepithelial sodium (and water) movement and does not appear to be specifically regulated. In contrast, specific transport processes partake in the control of K movements in the colon, where both K reabsorption and K secretion occurs. Colonic K secretion is subject to regulation by factors similar to those within the renal epithelium. However, the capacity of colonic K secretion is limited, and the kidney excretes the bulk of K.

The fact that by far the largest fraction of the body's K is located in cells with finite K permeabilities poses a constant threat to the maintenance of extracellular fluid K levels at low millimolar concentrations. As shown in Figure 2, active Na-K-ATPase activity is primarily responsible for effective K uptake into cells, and it is the balance between active Na-K activity and passive backleak that ultimately determines the transcellular distribution of K. Figure 3 provides information on several factors involved in the protective redistribution of K between cellular and extracellular fluid compartments. Shifts of K between extra- and intracellular fluid are important because they respond rapidly and are responsible for the prevention of harmful fluctuations of extracellular K. It is the coordinated interaction between K redistribution (internal K balance) and effective renal K excretion (external K balance) that safeguards K homeostasis.

General properties of the excretion of K

Clearance studies in the late 1940s and the 1950s provided the framework for our understanding of the basic mechanisms of renal K transport. By carefully comparing the amount of filtered K with excreted K, Berliner, Kennedy, and Orloff postulated that most freely filtered K is reabsorbed along the proximal tubule and the thick ascending limb (TAL) and that such K reabsorption precedes K secretion in more distally located tubule segments.[2] The observation that the excreted amount of K could exceed the amount of K filtered was the key observation, consistent with the presence of K secretion along the nephron. Evidence was also advanced to suggest that extensive K reabsorption precedes K secretion, and that the distal K secretion involves exchange for sodium (Na).

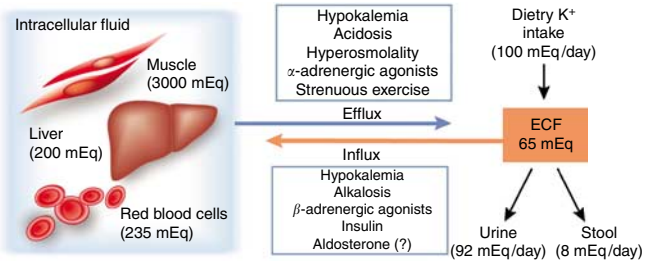

Figure 3 | Distribution of K^+ between the intracellular and extracellular fluid compartments. Only about 2% of the body's K^+ is present in the extracellular fluid, with most of the remainder in intracellular fluid, represented here by three of the largest cellular compartments. The distribution of K^+ represents a balance between active uptake to cells by the Na K^+-ATPase and the passive leak of K^+ from cells. This balance is influenced by the K^+ concentration of the extracellular fluid as well as by the factors listed above and below the two horizontal arrows. A typical daily K^+ intake of 100 mmol is matched by a small excretion in the stool plus the regulated excretion of K^+ by the kidneys. ECF, extracellular fluid.

Thus, K excretion was thought to result from simultaneous (proximal) reabsorption and (distal) secretion of K, with variable and controlled secretion being the key mechanism of K excretion. Further clearance studies explored the mechanism of K adaptation and the effects on K secretion of adrenal steroids, acid–base disturbances, and diuretics.

Tubule sites of K transport and its regulation

Figure 4 provides a summary of K transport along the mammalian tubule. Results are based on free-flow micropuncture experiments in which tubule fluid samples were collected from identified superficial nephrons and analyzed for potassium and inulin. It is apparent that the bulk of filtered K is reabsorbed by two segments—the proximal tubule and the thick ascending limb of Henle's loop—and that K is secreted along the initial and cortical collecting tubules. Recent studies have extended our knowledge of the location of K secretion to include an important contribution of the connecting tubule to the process of K secretion.

Three aspects of K transport deserve mention. First, as shown in Figure 4, K reabsorption also occurs along the very same nephron segments that secrete K. This process, which has been identified in animals exposed to a low-K diet, also takes place along the medullary and papillary collecting ducts. Second, a careful analysis of morphological and functional data provides strong evidence for functional heterogeneity of distal tubule segments such that principal cells mediate K secretion, whereas intercalated cells are responsible for K reabsorption. Finally, most of the factors known to modulate K excretion do so by changing K secretion. K secretion is stimulated by high K intake (K adaptation), during metabolic alkalosis, following administration of mineralocorticoids, and during increased distal delivery of Na. Thus, most diuretics induce tubule flow–dependent stimulation of K secretion (and excretion), an effect best explained by high distal tubule delivery rates of Na. The observed inhibition of distal K secretion following administration of amiloride, a potent apical Na channel blocker, underscores the importance of Na reabsorption for effective K secretion.

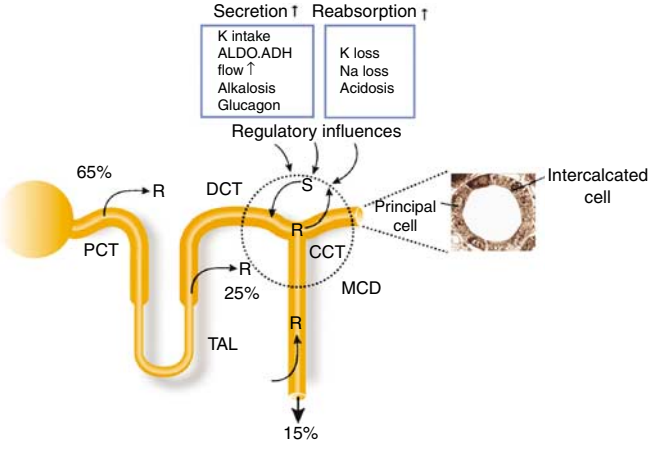

Figure 4 | Summary of potassium transport along the nephron. Following filtration, potassium is extensively reabsorbed along the proximal tubule and Henle's loop. Potassium is secreted along the initial and cortical collection tubules. Net secretion can be replaced by net absorption in a state of potassium depletion. Also shown are the two cell types lining the distal tubule and cortical collecting duct. ADH, antidiuretic hormone; ALDO, aldosterone; CCT, cortical collecting tubule; DCT, distal convoluted tubule; ICT, initial collecting tubule; MCD, medullary collecting duct; PCT, proximal tubule; R, reabsorption; S, secretion; TAL, thick ascending limb.

Cell models of K transport

A combination of methods—single-nephron transport studies and measurements of electrical profiles across defined tubule segments, cell K concentrations, and the fine structure of tubule cells—permits considerable insight into transport properties of K along the nephron (Figure 5).

K. reabsorption along the proximal tubule is extensive and depends critically on sodium reabsorption (Figure 5a). Two mechanisms, diffusion and solvent drag, have been proposed. Diffusion of K from lumen to peritubular fluid can be driven by the lumen-positive potential along the second half of the proximal tubule and the modest increase in lumen K concentration that has been occasionally observed. Solvent drag depends on Na transport, which generates local hypertonicity in the (unstirred) paracellular compartment, driving water reabsorption that entrains K (low reflection coefficient of K) and at the same time depletes the intercellular space of K. This process would additionally favor K diffusion from the lumen into the interspaces, and may even account for 'apparent' uphill transport of K across the proximal tubule epithelium. There is no evidence for specific regulation of K reabsorption along the proximal tubule, and most observed modulation of proximal K reabsorption can be accounted for by alterations in Na transport.

Thick ascending limb of Henle's loop

Figure 5b shows two parallel transport routes of K reabsorption: electroneutral K reabsorption by coupled Na-2Cl-K transport across the apical membrane and passive paracellular K transport driven by the lumen-positive potential. K transport across the basolateral membrane occurs by diffusion and potassium chloride (KCl) cotransport. It should be noted that K channels in the apical membrane are important because they permit recycling of K necessary for supplying K to the cotransport mechanism (not shown). Moreover, apical K channels are also critically involved in the generation of the lumen-positive potential that drives K reabsorption through the paracellular space. The role of apical K channels in TAL function is strongly supported by the clinical observation that significant K and Na loss occurs in a subgroup of patients with Bartter's syndrome, a genetic disorder in which apical K channels are absent.

Principal cells

Principal cells, located in the initial and cortical collecting tubules as well as in the connecting tubule, are the main site

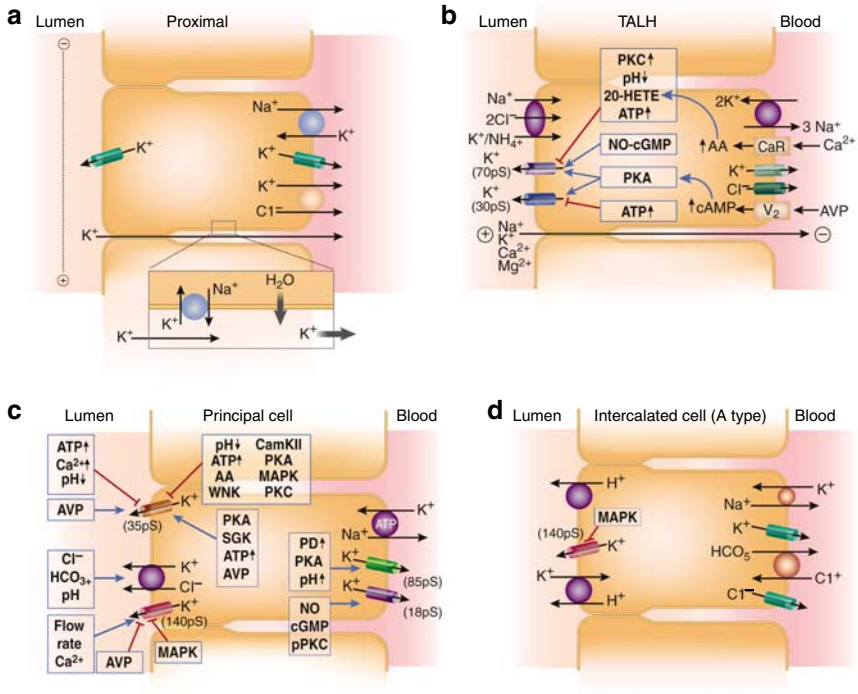

Figure 5 | Cell models of K transport along the nephron. Note the similarity of transporters in the basolateral membrane and widely differing transporters in the apical membrane. Both apical and basolateral transporters are affected by a large number of cellular and extracellular messengers. AVP, arginine vasopressin; CaMKII, calcium/calmodulin-dependent protein kinase II; cGMP, cyclic guanosine monophosphate; 20-HETE, 20-hydroxyeicosatetraenoic acid; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; SGK, serum glucocorticoid regulated kinase; TALH, thick ascending limb of Henle's loop; WNK, with no lysine (K).

of regulated renal K secretion. Figure 5c shows that secretion of K involves two steps: active uptake across the basolateral membrane, followed by passive movement across the apical membrane. The latter involves diffusion through K channels and electroneutral KCl transport. Na channels in the apical membrane are an additional component of effective K secretion because they depolarize the apical membrane. A modest amount of K may also enter the lumen through the paracellular pathway, driven by the strongly negative lumen-negative potential.

Intercalated cells

A subfamily of intercalated cells in the initial, cortical, and medullary collecting ducts reabsorbs K by an active, ATPase-dependent exchange process (Figure 5d). The activity of this electroneutral transporter becomes apparent in states of K depletion and may play a role in lowering urine K levels below plasma K concentrations. The enhancement of K-mediated hydrogen (H) secretion could also play a role in contributing to the metabolic alkalosis of K depletion. Metabolic acidosis and Na deprivation also enhance pump-mediated K-H exchange.

Regulation of K excretion

Proximal tubule urinary K excretion is not specifically affected by modulation of K reabsorption along the proximal tubule, and transport inhibitors of K-H exchange do not reduce K reabsorption. However, diminished reabsorption of fluid along the proximal tubule, for instance, following an increase in glucose load, administration of an osmotic diuretic such as mannitol, or reduction of bicarbonate reabsorption by carbonic-anhydrase inhibitors, can lead to increased sodium and fluid delivery into the distal tubule and to enhanced K secretion. When sodium reabsorption along the proximal tubule is enhanced, distal K secretion may be suppressed owing to diminished sodium and fluid delivery.

K channels are present in both apical and basolateral membranes of proximal tubule cells. These channels contribute importantly to the cell negativity, an important driving force for passive basolateral extrusion of chloride and bicarbonate.

Basolateral K channels are also activated by cell swelling, and their activity increases with stimulation of basolateral Na/K turnover. Apical K channels are also stimulated by membrane stretch and membrane depolarization, as well as elevation of cell calcium. Activation of apical K channels thus tends to minimize the decline of the apical membrane potential whenever electrogenic sodium reabsorption rises (for example, during elevation of sodium-dependent glucose or amino-acid transport). This response minimizes changes in the driving force for the apical entry of Na with organic solutes.

Thick ascending limb

Two apical K channels (the low-conductance ROMK and a 70-pS K channel) are involved in K recycling across the apical

membrane, and their modulation affects net reabsorption of NaCl. The activity of both channels is affected by several cell messengers (Figure 5b), by hormones such as vasopressin and aldosterone, by dietary changes of K, and by changes in external calcium (Ca) concentration. Although the TAL is normally a site of significant net reabsorption of K, the direction of transport can be reversed following inhibition of Na-2Cl-K cotransport (loop diuretics).

It should be noted that the apical cotransporter site for K has significant affinity for ammonium ions. Competition between K and ammonium in the fluid entering the TAL may underlie the reciprocal relation between urinary K and NH_4 excretion. In hypokalemia (low K in the fluid entering the TAL), increased H secretion in collecting ducts may result from preferential NH_4 transport into the renal medulla interspaces. In hyperkalemia (high K in the fluid entering the TAL), ammonium accumulation in the renal medulla and its subsequent secretion into collecting ducts may be compromised by reducing NH_4 reabsorption in the TAL.

Principal cells

Three pathways for K transport participate in the translocation of K across the apical membrane (Figure 5c). They include two K channels—ROMK and maxi-K channels—and KCl cotransport. An important fraction of apical K secretion is mediated by ROMK channels, but maxi-K channels have emerged recently as a second transport pathway responsible for K secretion. Maxi-K channels mediate K secretion particularly when distal tubule flow rate and Na delivery increase, following luminal application of physiological concentrations of vasopressin, and during administration of a high-K diet. Changes in blood aldosterone levels and in dietary intake of K are also important factors that modulate K secretion. Changes in apical endocytosis have recently been shown to participate in the regulation of ROMK channels following alterations in dietary K. K secretion through apical KCl cotransport is normally modest but may increase following reduction in lumen Cl concentrations during delivery of poorly reabsorbed anions (bicarbonate, phosphate) into the connecting and collecting ducts.

An interesting feature of K transport regulation in principal cells is the coordination of apical and basolateral transport elements. The effects of aldosterone and high K intake are representative examples. Aldosterone stimulates K secretion not only through activation of apical Na channels but also by elevating basolateral Na-K activity, by augmentation of K permeability in both apical and basolateral membranes, and, when chronically administered, by stimulating basolateral membrane growth. The same transformation of principal cell function and structure follows an increase in K intake. It is also of interest that stimulation of basolateral Na-K activity by acutely raising basolateral K not only invokes basolateral electrogenic pump activation but also enhances apical Na and K permeabilities. The mechanism responsible for 'crosstalk' between apical and basolateral membranes is incompletely understood.

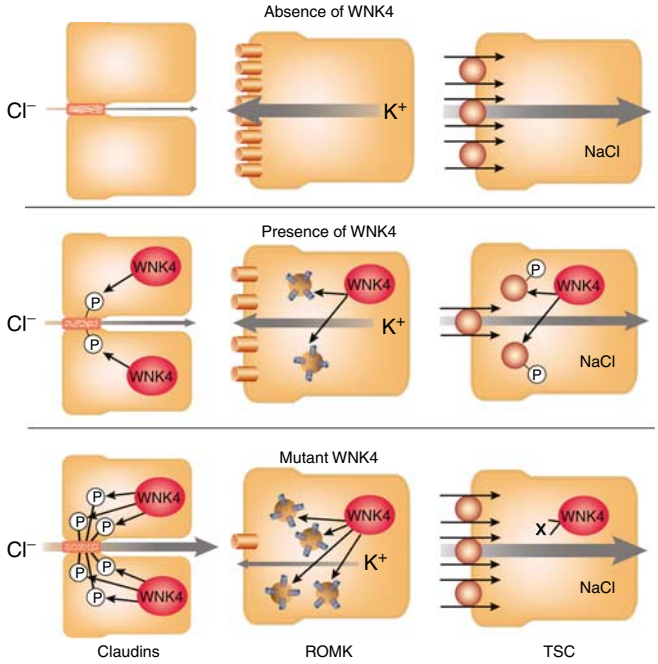

Figure 6 | Summary of the proposed effects of WNK4. Note the three components of action: (i) inhibition of NaCl transport (in the distal convoluted tubule), (ii) inhibition of K secretion (in principal tubule cells), and (iii) stimulation of paracellular chloride conductance. Mutations in WNK4 enhance NaCl cotransport and paracellular chloride conductance and block K secretion, leading to pseudohypoaldosteronism type II.[68] ROMK, renal outer medullary potassium channels.

Competition between K and NH_4 may also occur at the basolateral K binding site of Na-K-ATPase and Na-2Cl-K cotransport in the medullary and cortical collecting ducts. Such competition may partly explain the observation that high K in the peritubular fluid diminishes NH_4 secretion, whereas the opposite has been observed during K depletion. High ammonium levels have also been shown to interfere with effective K excretion.

An interesting recent development concerns the role of WNK—with no lysine (K)—kinases in the regulation of K and Na transport (Figure 6). These kinases affect both Na and K cotransport in the distal convoluted tubule and apical ROMK channels in principal cells by reducing NaCl cotransport and apical ROMK activity. Stimulation of WNK would thus be expected to lead to increased delivery of Na to connecting and collecting tubules, yet curtail K secretion.

Intercalated cells

Studies of the regulation of apical H-K cotransport have focused largely on the effects of changes in acid–base, Na, and K balance. As expected, low K and chronic acidosis stimulate H-K exchange and upregulate carrier expression. Administration of a low-Na diet has a similar effect. Several isoforms of K-H exchange (gastric and colonic) have been identified in renal tubules, and they differ with respect to inhibitor sensitivity and ionic specificity. Some uncertainty of the

physiological role of H-K exchange concerns the finding that knockout of specific isoforms of H-K-ATPases fails to induce robust changes in urinary K excretion, especially following administration of a low-K diet.

TRANSPORT MECHANISMS OF POTASSIUM

Chair: Steven C. Hebert
Yale University School of Medicine, USA

This session focused on key membrane transporters involved in membrane transport of potassium and provided an overview of the transport pathways of potassium at the main sites of tubule potassium secretion in the kidney.

Torben Clausen (Institute of Physiology, Denmark): 'Properties and Regulation of Na^+-K^+-ATPase.' Sodium-potassium ATPase (Na^+-K^+-ATPase; Na^+-K^+ pump) is located in plasma membranes and mediates the active extrusion of sodium and accumulation of intracellular potassium.[4] Clausen discussed the importance of tight regulation of Na^+-K^+-ATPase in muscle that contains the largest amount of this important Na^+-K^+ pump and the crucial nature of its tight regulation for the maintenance of plasma potassium, membrane potential, and the excitation-contraction of muscle. Muscle excitation-contraction is associated with a rapid influx of sodium and an equivalent loss of potassium. The job of the Na^+-K^+-ATPase is to reclaim the loss of potassium and the cellular accumulation of sodium. Matching these ion losses with increased Na^+-K^+-ATPase activity is crucial for minimizing the loss of muscle excitability and the rate of muscle force decline due to increasing extracellular potassium. Fortunately, excitation increases Na^+-K^+-ATPase activity within seconds, so as to maintain muscle activity during intense exercise. This rapid increase in Na^+-K^+-ATPase activity in muscle is achieved not only by excitation but also by catecholamines, insulin, insulin-like growth factor-1 (IGF-1), calcitonins, and amylin. Long-term regulation of the sodium pump also involves multiple factors, including thyroid hormones, adrenal steroids, insulin, level of training or inactivity, and K balance.[5]

Gerardo Gamba (National Institute of Nutrition, Mexico): 'The Apical, Renal-Specific Na-K-2Cl Cotransporter.' This session continued the discussion of K transporters. The renal-specific Na-K-2Cl cotransporter (NKCC2) is expressed in apical membranes of the TAL and is a crucial component of the NaCl absorptive mechanism in this nephron segment. Consistent with its vital role in reabsorption of 20–25% of the filtered load of NaCl, loss-of-function mutations in this cotransporter give rise to the severe renal salt–wasting antenatal Bartter's phenotype (hyperprostaglandin E syndrome; Bartter's type I). Gamba discussed the molecular physiology of this cotransporter[6] with particular focus on its regulation by vasopressin (antidiuretic hormone), intracellular chloride and the WNK serine-threonine kinases. Six isoforms of NKCC2 are expressed in the kidney (mouse) due to the combination of two alternative splicing mechanisms. The first splicing involves a mutually exclusive cassette (termed A, B, and F) that encodes part of the second transmembrane span. These isoforms show a specific axial expression pattern along the TAL from medullary to cortical and exhibit differences in ionic affinities—this may explain the heterogeneity of salt-transport properties along the TAL. The second splicing event dramatically shortens and alters the amino acid sequence of the C-terminus of NKCC2; this 'short' form of NKCC2 has been implicated in the regulated trafficking of the cotransporter to the apical plasma membrane by vasopressin. Finally, Gamba discussed the recent advances[7] in our understanding of how two other serine-threonine kinases (PASK and WNK1 and WNK4) play important roles in modulating NKCC2 activity with changes in cell chloride concentration. The latter is vital to maintaining cell volume during rapid changes in NaCl transit through the TAL cell.

Thomas D. DuBose and Juan Codina (Wake Forest University School of Medicine, USA): 'The Molecular Regulation and Physiology of the H^+-K^+-ATPases in Kidney.' H^+-K^+-ATPase is expressed in the apical membranes of collecting-duct cells and mediates the active extrusion of protons in a one-for-one exchange for potassium. Both gastric ($HK\alpha_1$) and colonic ($HK\alpha_2$) types of H^+-K^+-ATPases are expressed in the kidney and differ in their sensitivities to the inhibitors Sch-028080 and ouabain.[8] The colonic form, $HK\alpha_2$, is most robustly upregulated (both mRNA and protein) by chronic hypokalemia, in which it serves to actively reabsorb potassium. DuBose described the details of the assembly and function of the α- and β-subunits that form the colonic H^+-K^+-ATPase. Specifically, he discussed the crucial role of the C-terminus of $HK\alpha_2$ in assembly and proper trafficking of this pump to the apical plasma membrane. Without this C-terminal-driven assembly the pump would be destined for intracellular destruction. DuBose also referred to recent studies that have highlighted the importance of C-terminal-dependent interaction with other proteins that may regulate expression or function of this potassium-dependent proton pump.[9] Specifically, he described the potential role of the adapter protein CD63 in molecular regulation of the α-β-heterodimer forming the H^+-K^+-ATPase. The possible pathophysiological roles of the H^+-K^+-ATPase in distal renal tubular acidosis (RTA) and in chronic metabolic alkalosis were also discussed. Deficiency of $HK\alpha_2$ is a potential, albeit unproven, candidate for distal RTA associated with severe hypokalemia. In contrast, enhanced activity of the colonic H^+-K^+-ATPase in response to hypokalemia may contribute to the maintenance of chronic metabolic alkalosis.[10]

Lawrence G. Palmer and Gustavo Frindt (Weill Medical College of Cornell University, USA): 'Routes of K^+ Secretion Across the Distal Nephron.' K secretion along the distal nephron and collecting duct contributes importantly to maintenance of K homeostasis. ROMK potassium channels mediate a significant component of net K secretion across apical membranes of aldosterone-sensitive distal nephron cells, including the distal convolute tubule segment 2,

connecting tubule, initial collecting duct, and cortical collecting duct (CCD).[11–13] However, evidence has accumulated for a significant participation of calcium-activated, maxi-K (bradykinin) potassium channels in distal K secretion at least under certain conditions (e.g., during high K intake and high tubule fluid flow rate). Palmer described studies that implicate a potential role for bradykinin channels in K secretion by intercalated cells following an increase in K intake. Membrane patches demonstrated the presence of bradykinin channels in intercalated cells but not principal cells. The classic mechanism for K secretion requires apical sodium entry via amiloride-sensitive sodium channels to depolarize the membrane potential and increase the driving force for K secretion, together with a mechanism for active entry of K across the basolateral membrane (e.g., via the Na^+-K^+-ATPase).[13] Neither of these pathways has been detected in intercalated cells. However, Palmer observed a significant amiloride-insensitive component of K excretion following a high K intake, implicating an additional unknown pathway for K secretion. Participation of bradykinin channels in this K-secretion process would require elevated cell calcium and a mechanism for basolateral K entry.

INTERNAL EXCHANGES AND RENAL REGULATION OF POTASSIUM BALANCE

Chair: Donald V. Seldin

University of Texas Southwestern Medical Center, USA

The second session of the symposium concerned a detailed examination of the internal exchanges and renal regulation of potassium balance.

Jang H. Youn and Alicia A. McDonough (University of California Keck School of Medicine, USA): 'Internal K Balance.' Youn and McDonough investigated the mechanisms by which internal K balance is maintained in the face of varied K intake and different humoral activities.[14,15] Because 90% of body K resides in muscle, clearly the movement of K across the muscle membrane is critical to maintain a fairly constant extracellular K despite wide swings in input. This in turn implies that the ultimate focus of internal K balance is the activity and regulation of the principal determinant of muscle K balance, the Na-K-ATPase ensemble.

By maintaining plasma K constant via a K clamp, Youn was able to show that in early K deprivation or during glucocorticoid treatment, K uptake in response to insulin was depressed (insulin resistance) while glucose uptake was normal and the pump was actually unchanged. With a background of a high-fat diet, insulin-stimulated K uptake was maintained, while insulin-stimulated glucose uptake was depressed.

An additional stimulus for K uptake was mediated by activation of AMP-dependent protein kinase, which would prevent the rise of plasma K during exercise. Evidence could not be advanced to support the hypothesis that a splanchnic K sensor plays a role in regulating K uptake or renal excretion.

Taken together, these studies indicate that under certain circumstances muscle uptake of glucose and K can be independently regulated by insulin; moreover, this regulation is not necessarily mediated by changes in Na-K-ATPase activity.

Steven C. Hebert (Yale University School of Medicine, USA): 'Renal K Regulation.' Hebert presented evidence that the ROMK channel is critical for maintaining K balance for the following reasons. First, in the TAL, K secretion from cell to lumen ensures a sufficient supply of K to permit electroneutral Na-K-2Cl transport. Second, the K secreted into the lumen along this channel also, in part, back-diffuses along the paracellular pathway, participating in a current loop that entails Cl basolateral exit. In the absence of apical ROMK channels in TAL, both processes are inhibited, thereby suppressing Na-K-2Cl reabsorption—type I Bartter's syndrome. Third, in the late distal tubule and CCD, the absence of ROMK channels leads to transient hyperkalemia in infants with Bartter's syndrome (this process is subsequently reversed, with enhanced K excretion and hypokalemia as a result of an adaptive appearance of flow-stimulated maxi-K channels).[16,17]

Gerhard Malnic (University of São Paulo, Brazil): 'Luminal Factors Regulating K Transport.' Malnic examined the regulation of cortical distal tubule transport using an *in vivo* stationary microperfusion method in rat and mouse kidney. By varying luminal composition and adding various inhibitors and stimulators, the mechanism of transport could be dissected.[18–20] The addition of arginine vasopressin stimulated K secretion, an effect mediated by stimulation of apical V1 receptors leading to an activation of a pathway involving increased protein kinase C activity and increased intracellular Ca, and thus activation of Ca-sensitive maxi-K channels.[18,19]

K secretion in the distal tubule is well known to be increased by non-reabsorbable anions, via increasing luminal negativity. An additional action is the stimulation of electroneutral KCl secretion by a low luminal Cl concentration. Finally, bicarbonate *per se* stimulates increased K secretion by an unknown mechanism, in addition to both its alkalinizing properties and effects on electrical potential.[20]

These studies show that a number of luminal factors influence distal tubule K secretion, including peptide hormones (vasopressin and angiotensin), prostaglandins, and impermeant anions. Data on the signaling path mediating peptide effects suggest that, besides ROMK channels, Ca-dependent maxi-K channels participate in distal K secretion. In addition, experiments with impermeant anions indicate that an electroneutral KCl cotransport may also be involved in this process.[19]

Wen-Hui Wang (New York University, USA): 'Superoxide Is Involved in the Regulation of ROMK Channel Activity.' The regulation of K excretion has been attributed largely to the effects of luminal composition (Na, reabsorbed anions, flow rate, etc.) as regulated in large part by hormonal mediators. Electrochemical gradients, particularly as determined by aldosterone and other humoral agents, have been assigned a prominent role in the regulation of apical ROMK secretory K channels. Wang has emphasized channel renewal

and insertion as an additional regulator in circumstances of changes in external potassium balance.[21,22]

On a low-K diet, phosphorylation of a critical channel site is a key element in stimulating endocytotic removal of apical ROMK channels. This phosphorylation process is activated by superoxide production in low-K states. This process can be reversed by inhibition of superoxide production despite the presence of K deficiency. Thus, it is possible that superoxide may be able to acutely regulate ROMK channel activity through modulation of protein-tyrosine kinase and protein-tyrosine phosphatase interaction. Since the major source of superoxide is from activation of nicotinamide adenine dinucleotide phosphate hydrogenase oxidase, the effect of low K intake on ROMK channel activity and renal K secretion in gp91(-/-) mice was studied. It has been shown that deletion of gp91 attenuates the low K-induced increase in superoxide and increases the renal K secretion in K-restricted animals. It is concluded that superoxide plays an important role in mediating the effect of low K on renal K secretion and ROMK channel activity.[23]

Alan Weinstein (Weill Medical College of Cornell University, USA): 'A Mathematical Model of Rat Distal Convoluted Tubule.' Weinstein has analyzed the important contribution to K balance of the cortical connecting tubule.[24] The site and importance of K regulation have usually been determined on the basis of studies of the collecting duct. Recently, an important role has been assigned to the connecting tubule. Weinstein has carried out modeling studies of the rat connecting tubule and attributes the following properties to this nephron segment. (i) Transport rates of Na and K are both fourfold greater than in the cortical collecting tubule. This higher intrinsic transport rate, combined with the 5-to-1 coalescing of connecting segments to collecting ducts, provides a full order-of-magnitude difference in maximal K secretion by these two segments. (ii) A critical difference between the connecting and collecting tubules derives from the lower water permeability of the connecting tubule. Hypotonicity of the connecting tubule luminal solution minimizes the magnitude of the adverse K gradient opposing K secretion. (iii) Water reabsorption under the impact of antidiuretic hormone has a dual effect: Na reabsorption increases, thereby stimulating luminal K secretion; on the other hand, with water reabsorption, tubule fluid K concentration rises to a high concentration, increasing passive K back-flux and thus counteracting the stimulatory effect of Na reabsorption to augment K secretion. (iv) K secretion in the connecting tubule is enhanced independently by entering flow and entering Na concentration.

THE KIDNEY IN K HOMEOSTASIS AND THE ROLE OF K IN GASTRIC AND COLONIC TRANSPORT AND IN CIRCULATION

Chair: Shigeaki Muto
Jichi Medical School, Japan

Lisa Satlin (Mount Sinai School of Medicine, USA): 'Developmental Aspects of K^+ Transport in the Cortical Collecting Duct (CCD). ' Two types of K^+ channels—the inwardly rectifying, ATP-sensitive secretory K^+ (SK) channel encoded by ROMK and the Ca^{2+}- and stretch-activated maxi-K^+ channel—have been identified in the apical membrane of the adult mammalian CCD. The SK/ROMK channels are thought to mediate baseline K^+ secretion, whereas the maxi-K^+ channels appear to participate in flow-stimulated K^+ secretion. The CCD plays a key role in K^+ secretion in the adult mammalian kidney, but K^+ secretion is absent during the first 3 weeks of life in rabbit kidney.[25] In patch-clamp analysis in the maturing rabbit CCD, the SK/ROMK channels were detected only after the third week of postnatal life and increased with advancing age. Similar developmental changes in ROMK mRNA and protein expression have been observed in the maturing rabbit CCD. In addition, flow-stimulated K^+ secretion by the maxi-K^+ channel increased only after 5 weeks of postnatal rabbit kidney development. This increase in flow-stimulated K^+ secretion correlated well with maxi-K^+ channel α-subunit mRNA and protein abundance.[26] Therefore, the K^+ retention characteristic of the neonatal kidney is due, at least in part, to a paucity of apical SK/ROMK channels and maxi-K^+ channels in the CCD early in life. The observation that the maxi-K^+ channel is localized predominantly to the apical membrane of intercalated cells raises the intriguing possibility that intercalated cells, traditionally not considered to play a role in urinary K^+ secretion, may directly or indirectly contribute to flow-stimulated net K^+ secretion.

Michael Caplan (Yale University School of Medicine, USA): 'The Mechanisms for the Sorting and Regulation of the Gastric H^+-K^+-ATPase and Na^+-K^+-ATPase.' These two pumps are closely related members of the P-type ATPase family, but they are sorted to opposite membrane domains in polarized epithelial cells, and their enzymatic activities are subject to distinct regulatory pathways. Under resting conditions, the gastric H^+-K^+-ATPase is localized in the cytoplasmic compartments, whereas activation of acid secretion results in the exocytic insertion of H^+-K^+-ATPase-rich tubulovesicles with the apical membrane. Caplan showed that the cytoplasmic N-terminal tail of the H^+-K^+-ATPase β-subunit contains a tyrosine-based endocytosis signal responsible for the cessation of acid secretion through the retrieval of the pump from the apical surface to the regulated cytoplasmic compartments. Furthermore, he demonstrated that the β-subunit of the H^+-K^+-ATPase interacts directly and specifically with CD63, which is a member of the tetraspan family and is present in the cytoplasmic compartments involving the H^+-K^+-ATPase in unstimulated gastric parietal cells.[27] This interaction is required for the rapid endocytosis of the H^+-K^+-ATPase β-subunit protein, because in the absence of CD63 the H^+-K^+-ATPase β-subunit accumulates at the plasmalemma, whereas the H^+-K^+-ATPase β-subunit coexpressed with CD63 is rapidly endocytosed and accumulates in the cytoplasmic compartments. In addition, several candidate interacting proteins responsible for the sorting and delivery

of the Na^+-K^+-ATPase to the basolateral surface have been discussed.

Richard Warth (University of Regensburg, Germany): 'K^+ Channels and Their Function in the Luminal and Basolateral Membranes of the Colonic Epithelial Cells.' In the basolateral membrane, two distinct K channels—the KCNN4 (IK1 or SK4) K^+ channel and the heteromeric KCNE3/KCNQ1 K^+ channel—have been identified at the molecular level.[28–31] The KCNN4 K^+ channels appear to underlie the important Ca^{2+}-activated basolateral potassium conductance of colonocytes, which is activated by cholinergic stimulation such as with carbachol. The KCNE3/KCNQ1 K^+ channels, together with the cystic fibrosis transmembrane conductance regulator Cl^- channel localized at the luminal membrane, are involved in the cyclic AMP-mediated Cl^- secretion in the colon. The luminal membrane of the colonic epithelial cells possesses maxi-K^+ channels (KCNMAl plus accessory β-subunits), which appear to be important as a luminal K^+ exit pathway.[31] They are activated by increases in cytosolic free Ca^{2+}, e.g., after purinergic receptor stimulation, and probably transcriptionally by aldosterone and a high-potassium diet. An increase in KCNMA1 open probability directly enhances fecal K^+ loss and influences colonic Na^+ and Cl^- transport.

Stuart Linas (Denver Health Medical Center, USA): 'Relation between Potassium and Hemodynamics.' The effect of K on vascular tone and the role of K in the management of hypertension and cardiovascular prevention in normotensive or hypertensive subjects have been extensively studied in the past 20 years.[32–34] Linas reviewed articles on these topics, including epidemiologic, interventional, and animal studies. On the basis of these studies, increases in dietary or supplemental K appear to be antihypertensive and warranted in the prevention of hypertension (and possibly stroke risk) and treatment of patients with high blood pressure, especially in those who are thought to be salt sensitive. The antihypertensive effect of potassium is associated with natriuresis and is mediated in great part by total body sodium depletion.

Steven Sansom (University of Nebraska Medical Center, USA): 'Maxi-K^+ Channels in Glomerular Mesangial Cells (MCs).' These contractile cells in the glomerulus, which control glomerular filtration rate (GFR) by regulating the surface area of the glomerular capillaries. MC excitability is modulated by a variety of vasoactive agents, such as angiotensin II, which increases cytosolic free Ca^{2+} levels.[35] MCs possess Ca^{2+}-activated maxi-K^+ channels, which are involved in regulating excitability of MCs. Angiotensin II induces contraction of MCs by raising cytosolic free Ca^{2+}. Repolarization of the cell membrane by maxi-K^+ channels would inactivate voltage-operated Ca^{2+} channels and reduce Ca^{2+} influx. This effect of maxi-K^+ channels would act as a brake on the counteracting effect of angiotensin II. The maxi-K^+ channels are activated by atrial natriuretic peptide, nitric oxide, and the second-messenger cyclic GMP, which activates protein kinase G, and are inhibited by protein phosphatase 2A–induced dephosphorylation. Mice lacking the gene encoding the β_1-accessory subunit of maxi-K^+ channels had a normal GFR under basal conditions, but they failed to elevate their GFR to the same extent as wild-type mice following acute volume expansion, an effect consistent with an important role for these channels in the regulation of GFR under the demanding conditions of volume expansion.[36]

PHYSIOLOGY AND PATHOPHYSIOLOGY OF RENAL K HANDLING

Chair: Robert Alpern
Yale University School of Medicine, USA

F. John Gennari (University of Vermont College of Medicine, USA): 'K Transport and Homeostasis in Chronic Renal Disease.' This talk reviewed factors regulating renal K^+ excretion, coupled with observations in chronic kidney disease (CKD) patients, and then proposed a new insight into the role of hyperkalemia in maintaining K^+ balance when renal function is impaired.[37] Regardless of the prevailing serum [K^+], K^+ balance must be maintained to sustain life. Although unable to rapidly excrete K^+, CKD patients in fact remain in K^+ balance in the steady state, even when given supplemental K^+ (ref. [38]). In contrast to individuals with normal renal function, however, CKD patients given extra K^+ develop a stable hyperkalemia. Because hyperkalemia is known to stimulate K^+ excretion independent of and additive to stimulation by aldosterone,[39,40] this observation suggests a causal link between hyperkalemia and reestablishment of K^+ balance. In isolated collecting tubules, an increase in bath [K^+] produces changes in epithelial cell voltage and conductance within seconds compatible with increased Na^+-K^+-ATPase activity and upregulation of both ROMK and the epithelial sodium channel (ENaC).[41]

In intact animals and humans, hyperkalemia also stimulates aldosterone secretion, further activating Na^+-K^+-ATPase and ENaC, an effect that acts in synergy with hyperkalemia to further increase K^+ excretion. Hyperkalemia in CKD has been ascribed to concomitant hypoaldosteronism, but the link between hyperkalemia and aldosterone deficiency is not clear. Aldosterone levels correlate directly with serum [K^+] in patients with CKD regardless of whether they are low or normal,[42] an effect opposite to that expected if hyperkalemia was caused by aldosterone deficiency.

Dr Gennari proposes that steady-state serum [K^+] in CKD is determined primarily by the degree of renal damage, dietary K^+ intake, and the prevailing aldosterone level. According to this proposal, hyperkalemia is an adaptive response and, in a sense, the final 'safety valve' that provides the stimulus for the necessary K^+ excretion needed to effect K^+ balance.[37] CKD may also provide insight into K^+ adaptation. When placed on a high-K^+ diet, animals and humans increase K^+ excretion independent of aldosterone and distal Na^+ delivery. Serum [K^+] rises transiently in humans after high-K^+ meals. Repeated transient increases may not only increase K^+ excretion acutely but also produce

an increase in basolateral membrane surface area and upregulation of transport characteristic of K^+ adaptation. From a clinical perspective, patients with CKD and hyperkalemia should be managed primarily by ensuring that dietary K^+ intake is regulated, rather than by acute interventions to lower serum $[K^+]$.

Susan Wall (Emory University School of Medicine, USA): 'Transport of NH_4^+ in the Kidney.' Wall focused on the interactions between K^+ and NH_4^+ transport. Ammonium excretion is the major regulated component of net acid secretion. For more than 50 years the mechanism of ammonium excretion was thought to occur through active H^+ secretion in parallel with the nonionic diffusion of NH_3. However, direct transport of NH_4^+ is an important component of net acid secretion.

NH_4^+ and K^+ have a similar hydrated radius and are transported through many common pathways, such as through K^+ channels, NKCC1, NKCC2, and the Na-K-ATPase. K^+ and NH_4^+ are competitive inhibitors for binding sites on many transporters. Thus, increased interstitial K^+ concentration limits NH_4^+ uptake on these transporters, which may attenuate secretion of net H^+ equivalents. For example, across the basolateral membrane of the rat terminal inner medullary collecting duct, uptake of NH_4^+ through the Na-K-ATPase with NH_3 efflux generates an NH_3 'shuttle', which provides the cell with a source of H^+ for luminal secretion. Since K^+ and NH_4^+ are competitive inhibitors for a common extracellular binding site on the Na-K-ATPase, NH_4^+ uptake is inhibited by increased extracellular K^+ concentration or through the addition of ouabain, which results in reduced net acid secretion.[43–46]

Alternatively NH_4^+/K^+ uptake can be coupled to the transepithelial transport of a third ion. For example, NKCC1 functions as a Na^+-K^+(NH_4^+)-Cl^- cotransporter that participates in the transepithelial transport of Cl^- in the rat outer medullary collecting duct. Uptake of K^+ (or NH_4^+) and Cl^- are coupled to the inwardly directed Na^+ gradient.[45] NH_4^+ and K^+ are recycled across the basolateral membrane, whereas Cl^- is secreted across the apical membrane into the luminal fluid. Recent evidence suggests that some proteins, such as the blood group Rh proteins RhBG and RhCG, mediate transport of NH_4^+ but not K^+, at least in heterologous expression systems. It remains to be determined whether RhBG and/or RhCG mediate net acid secretion in native renal tissue.[46]

K^+/NH_4^+ transport pathways contribute significantly to net acid secretion and the transepithelial transport of other ions along the nephron. Owing to these transport mechanisms, changes in K^+ homeostasis can have significant effects on NH_4^+ secretion in the kidney.

Laurent Schild (University of Lausanne, Switzerland): 'Steroid Effects on Renal K^+ Transport.' The distal nephron, from the distal convolute tubule to the outer medullary collecting duct, is potassium- and aldosterone-responsive.[47–49] Aldosterone induces many of its effects through the induction of transcription and the generation of aldosterone-induced transcripts. One of these, SGK, is induced rapidly, leading to detectable increase in protein within 2 h. Whereas Sgk1 enhances the abundance of ROMK channels in Xenopus oocytes, aldosterone does not increase ROMK abundance in isolated CCDs. In cultured mouse CCD cells, aldosterone applied overnight increases transepithelial Na^+ transport but does not induce barium-sensitive K^+ currents. Thus, aldosterone-induced increases in transepithelial K^+ secretion are likely due to changes in driving force.[11–13]

Richard Lifton (Yale University School of Medicine, USA): 'Genetic Derangements of Renal K^+ Transport' Lifton discussed WNK kinases that function as molecular switches that regulate NaCl and K^+ homeostasis.[50,51] A central question in biological regulation is how complex, interdependent systems coherently respond to perturbation to reestablish normal homeostasis. This is highlighted in the kidney, where myriad channels, cotransporters, and paracellular flux pathways serve to ultimately maintain precise control of fluid and electrolyte homeostasis. Human diseases in which this normal homeostasis is lost provide the opportunity to identify key elements of these regulatory pathways.

One such disease is pseudohypoaldosteronism type II (PHAII), sometimes referred to as the syndrome of hypertension plus hyperkalemia, or Gordon's syndrome. PHAII is characterized by hypertension and hyperkalemia due to a renal secretory defect despite normal GFR; impaired distal renal proton secretion with consequent acidosis is a variable finding. This disease shows autosomal dominant transmission, affording the opportunity to use molecular genetic approaches to identify the underlying disease gene(s). By identification and clinical characterization of patients and families with PHAII from around the world, genes for PHAII were first mapped to chromosomes 1, 12, and 17. Further investigation has to date identified two of these disease-causing genes, *Wnk1* and *Wnk4* (refs. 50, 51). Both are members of a novel family of serine-threonine protein kinases characterized by the absence of a highly conserved lysine residue in the catalytic domain. Wnk1 mutations that cause PHAII are large (20 000–45 000 base pair) deletions in the first intron that cause increased expression of the gene. Mutations in Wnk4 are missense mutations that are highly clustered in a conserved, negatively charged segment adjacent to the kinase domain.

Immunolocalization of Wnk1 and Wnk4 using specific antibodies has shown that these proteins localize to the distal convoluted tubule and collecting duct; in addition, they are found outside the kidney in a number of chloride-transporting epithelia, including pancreatic and hepatic bile ducts, colonic crypts, choroids plexus, and lung.

Insight into the functions of wild-type and mutant Wnk4 has come from *in vitro* studies. Wild-type Wnk4 is a potent inhibitor of the thiazide-sensitive Na-Cl cotransporter (NCC) when analyzed by coexpression in Xenopus oocytes. This inhibition is mediated by altered trafficking of NCC, with Wnk4 causing diminished cell-surface expression. This

inhibitory activity requires the kinase activity of Wnk4. Importantly, the missense mutations that cause PHAII abrogate inhibition of NCC, resulting in increased NCC activity; *in vivo*, this effect is expected to result in increased NaCl reabsorption, thereby accounting for the increased blood pressure seen in patients with PHAII. The other major pathway for Na+ reabsorption in the distal nephron is via the ENaC. This step is electrogenic, providing a lumen-negative potential that is used to drive either paracellular Cl^- reabsorption or transcellular secretion of K^+ (via the channel ROMK). *In vitro* studies indicate a role for Wnk4 in regulation of both processes. As for NCC, wild-type Wnk4 is a potent inhibitor of ROMK activity by diminishing its cell-surface expression. In contrast to its effect on NCC, however, PHAII-Wnk4 shows *increased* inhibition of ROMK, consistent with this effect's playing a role in the impaired K^+ secretion seen in patients with PHAII. Finally, Wnk4 also has a role in regulating paracellular Cl^- flux. MDCKII monolayers with inducible expression of wild-type Wnk4 show a selective increase in paracellular Cl^- flux owing to increased Cl^- permeability when Wnk4 is induced; moreover, PHAII-Wnk4 shows about a fivefold greater increase in absolute Cl^- permeability.

Together these findings indicate that Wnk4 is an integrator of diverse electrolyte handling pathways that acts to differentially regulate flux mediators to achieve specific physiologic ends. The mutations that define PHAII abolish inhibition of NaCl reabsorption via NCC, increase paracellular Cl^- flux, and increase inhibition of K^+ secretion. These functions suggest a solution to a paradox in renal physiology, the fact that aldosterone is secreted in two distinct physiologic states: in response to intravascular volume depletion with activation of the renin-angiotensin system and in response to hyperkalemia. This raises the question of how the kidney responds appropriately to aldosterone under these two distinct states, maximizing renal NaCl reabsorption in the former while maximizing renal K^+ secretion in the latter. Wnk4 appears to be a molecular switch that is poised to promote precisely the response desired in the former condition, simultaneously maximizing renal NaCl reabsorption via effects on NCC and paracellular Cl- flux while inhibiting K+ secretion. We presume that the clustering of PHAII mutations define a component of the switch itself, and that the mutations mimic a normal event *in vivo*, perhaps binding of an ion (Ca^{2+}?) to the negatively charged segment. These observations identify new regulators of ion transport and have implications for understanding mechanisms of achieving and maintaining fluid and electrolyte homeostasis.

PATHOPHYSIOLOGY OF K HOMEOSTASIS

Chair: Giovambattista Capasso
Second University of Naples, Italy

The final session focused on several topics of the pathophysiology of potassium homeostasis and included a presentation of recent insights into potassium channel trafficking and its implication for the regulation of K transport.

Mitchell Halperin (University of Toronto, Canada): 'Control of K Excretion *In Vivo*.' Although many factors may be implicated, it is not clear when each might play a critical role. The objective was to understand the regulation of K^+ excretion in humans. Hence, the experimental design was to mimic Paleolithic conditions when fundamental controls developed.[52] It is crucial to recognize that our present understanding is based on studies in rats with very high intakes of sodium and K^+ per kilogram of weight; this form of analysis adjusts for storage of dietary K^+ prior to excretion. In rats fed a low-electrolyte diet, administration of mineralocorticoids, NaCl, $NaHCO_3$, or mannitol, alone or in combination, increased distal flow rate, but there was little effect on K^+ excretion. In contrast, K^+ excretion rose markedly following an acute KCl load. The time course was critical. There was an immediate, near-maximal rise in the luminal $[K^+]$ in the terminal cortical collecting duct ($[K^+]_{CCD}$), but the kaliuresis was only modestly increased. Over the subsequent 4 hr, kaliuresis increased markedly, attributable solely to a higher cortical distal flow rate, which appeared to be due to diminished reabsorption of NaCl in Henle's loop. Dr Halperin speculates that there is a stepwise sequence in the control mechanisms; the initial step inserted luminal K^+ channels in the CCD, raising the $[K^+]_{CCD}$. Thereafter, an increase in the flow rate in the CCD was the major form of regulation. Thus, the conditions imposed by the experimental setting 'select' which of the possible control mechanisms might be revealed.

David Young (University of Mississippi, USA): 'The Role of Potassium in Cardiovascular Protection.' Increases in potassium concentration in the range expected to result from raising intake from a low to high-normal range can affect the cells of the vascular system and the myocardium in ways that may provide an explanation for the vascular and cardiac protective effects of diets with a high potassium content. One of the earliest events contributing to formation of the atherosclerotic lesion is oxidation of low-density lipoprotein cholesterol in the subintima of the arteries by reactive oxygen species produced by monocytes, macrophages, and possibly endothelial cells and smooth muscle cells. In view of the inhibitory response to elevation of potassium concentration of free radical formation by monocytes and endothelial cells reported by McCabe *et al.*,[53] elevation of potassium concentration may be effective in inhibiting the path toward lesion development at this proximal point. Vascular smooth muscle cell migration and proliferation in the subintima are processes involved in maturation of the lesion, and the findings of Ma *et al.*[54] of an inverse relationship between potassium concentration and migration and proliferation suggest that potassium's protective actions may include inhibition of the participation of smooth muscle cells in lesion formation as well. Potassium's ability to inhibit the thromboembolic consequences of advanced atherosclerotic lesions is supported by evidence from the studies of Lin and Young[55] that show an inhibition by elevation of potassium concentration of thrombus formation in the coronary and

carotid arteries of experimental animal models. These actions may provide protection against cardiovascular diseases that are related to lipid infiltration, foam cell formation, and accumulation in the subintima, as well as thrombus and embolus formation. Fitzovich *et al.*[56] observed that moderate potassium depletion induced by thiazide diuretics together with high sodium intake strongly impaired cardiac mechanical function in anesthetized dogs. The variables affected most significantly were those associated with the process of active relaxation that occurs during diastole. In healthy young adults, Srivastava and Young[57] reported that moderate potassium depletion of 7 days' duration induced by diuretics and high sodium intake also significantly impaired diastolic function, as estimated by peak rate of flow through the mitral valve. The observed effect on diastolic function in these studies of normal humans and animals may be of clinical importance in patients with heart failure. The findings presented here, along with others demonstrating inverse relationships between potassium intake and blood pressure, cardiac arrhythmias, and thromboembolic strokes in humans and animal models, provide emphatic support for the potential importance of high dietary potassium intake as a means for reducing risk of cardiovascular diseases.

Robert Unwin (University College London, UK): 'Tubular Acidosis and Renal K Transport.' Unwin's talk included a brief summary of what is known about kidney's response to acute versus chronic acidosis in terms of K excretion: acute acidosis causes retention (first described by Robert Berliner and colleagues), whereas chronic acidosis causes increased excretion, secondary to increases in sodium delivery and tubule fluid flow rate at the potassium-secreting distal nephron. The decrease in K secretion that underlies the reduction in urinary excretion in acute acidosis is related (in part) to the pH sensitivity of the apical ROMK potassium channel and changes in Na-K-ATPase activity, hydrogen ion secretion, and the transepithelial potential difference. A slight conundrum is the hypokalemia of the clinical disorder of RTA, in which hypokalemia is common, especially in the distal nephron (so-called 'classic' or type 1) form. The puzzle is that these patients remain hypokalemic even after correction of their acidosis or if they are not actually acidotic (the so-called 'incomplete' form of distal RTA). They seem to have an underlying 'leak' of K from the kidney. Although this might be explained partly by alkali therapy itself (although not in the 'incomplete' cases), mild secondary hyperaldosteronism, and tubular damage from associated nephrocalcinosis, these explanations seem insufficient. A recent finding is that a membrane transport protein or channel functions in a different way (mode), depending on its structure and local environment. The red blood cell expresses a key structural and functional protein, AE1, also known as 'band 3', which exchanges chloride (Cl^-) for bicarbonate (HCO_3^-). This protein is shared (in a truncated form) with the alpha-intercalated acid-secreting cells of the collecting duct. In at least one form of inherited distal RTA it is mutated and the cause of the renal acidification defect (although the red cells seem normal; see below), which may be due to mistargeting of the mutant AE1 from its normal site in the basolateral membrane to the apical membrane.[58] AE1 mutations are also found to be the cause of some inherited defects in red cell morphology, such as hereditary eliptocytosis (about 20% of cases). Indeed, it has even been reported that hereditary eliptocytosis can be associated with distal RTA. But the strange finding has been made that in another red cell abnormality, hereditary stomatocytosis, AE1 mutations can also be the cause and that these red cells 'leak' potassium (and can result in pseudohyperkalemia if a blood sample is left at room temperature for any length of time). When expressed in oocytes, these mutations are permeable to K and to Na.[59] The obvious questions arise: Could these AE1 mutants also 'leak' K in the collecting duct if they are expressed apically? Do the mutations found in AE1 in distal RTA also have this 'leak' property for K and Na? The answers to these questions are actively being sought.

Florian Lang (Institute of Physiology, Germany): 'The Role of SGK in Renal Sodium and K Transport.' The serum- and glucocorticoid-inducible kinase SGK1 was originally cloned as a glucocorticoid-sensitive gene from rat tumor cells and later as a human cell-volume-sensitive gene upregulated by osmotic and isotonic cell shrinkage.[60] Within the kidney SGK1 is expressed in the aldosterone-sensitive distal nephron. SGK1 increases ENaC in part by phosphorylation of the ubiquitin ligase Nedd4-2, which ubiquitinates ENaC, thus preparing the channel protein for clearance from the cell membrane and subsequent degradation. Phosphorylation of Nedd4-2 by SGK1 reduces the affinity of the enzyme to the target protein and thus disrupts the ubiquitination of ENaC, leading to enhanced ENaC channel protein abundance in the cell membrane. Besides ENaC, SGK1 enhances the activity of further renal transport systems, including the apical K^+ channel ROMK and the K^+ channel KCNE1. Gene-targeted mice lacking SGK1 ($sgk1^{-/-}$) show normal Na^+ excretion and blood pressure under normal salt intake.[61] The phenotype of the SGK1 knockout mouse became apparent only after exposure of the mice to a salt-depleted diet. NaCl excretion decreased under NaCl depletion in both $sgk1^{-/-}$ and $sgk1^{+/+}$ mice. However, the renal NaCl loss was significantly larger in $sgk1^{-/-}$ mice than in $sgk1^{+/+}$ mice, despite exaggerated increase of plasma aldosterone concentrations, decrease of blood pressure, decrease of GFR, and enhanced proximal tubular Na^+ reabsorption in the $sgk1^{-/-}$ mice.

In contrast to the mild phenotype of the $sgk1^{-/-}$ mouse, targeted disruption of the mineralocorticoid receptor in mice leads to severe salt wasting, even at normal salt intake, and the ENaC knockout mouse is not viable. Thus, SGK1 is not required for the function of ENaC but significantly contributes to—yet does not fully account for—the effects of mineralocorticoids in the kidney. During sufficient salt supply, the enhanced plasma aldosterone concentration of the $sgk1^{-/-}$ mouse overrides the lack of SGK1 and allows the maintenance of normal blood pressure. Despite increased plasma aldosterone concentrations, the plasma K^+ concen-

trations were higher in $sgk1^{-/-}$ than in $sgk1^{+/+}$ mice, pointing to a deficit in renal K^+ excretion. Subsequent clearance studies indeed disclosed a moderate impairment in the ability of $sgk1^{-/-}$ mice to eliminate acute and chronic potassium loads.[62] The effect may be partially due to the lack of stimulation of the renal outer medullary K^+ channel ROMK1 by SGK1 and/or an additional K^+ channel. More importantly, SGK1-dependent activity of ENaC is expected to depolarize the apical cell membrane of principal cells, thus favoring K^+ secretion. Considering the significance of renal Na^+ transport for blood pressure regulation, the effects of SGK1 on ENaC were expected to influence blood pressure. A role of SGK1 in blood pressure regulation became apparent during high-fat and high-salt diets, which have both been shown to promote increased blood pressure. Exposure of mice on a standard diet to saline in drinking water did not lead to differences in blood pressure between $sgk1^{-/-}$ and $sgk1^{+/+}$ mice. A high-fat diet increased blood pressure to a similar degree in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice, an observation that, again, indicates the participation of SGK1-independent mechanisms. However, the additional salt load further increased blood pressure in $sgk1^{+/+}$ but not in $sgk1^{-/-}$ mice, which demonstrates the role of SGK1 in hypertension during intake of excessive fat and salt. A high-fat diet leads to increased insulin plasma concentrations, which in turn are expected to stimulate SGK1 and subsequently ENaC. Obesity is associated with peripheral insulin resistance, abnormal glucose metabolism, and hyperinsulinemia; the latter favors the development of salt-sensitive hypertension in obese subjects by increasing salt and water reabsorption on different nephron segments. Along those lines, enhanced SGK1 expression has been observed in the salt-sensitive Dahl rat. In addition, moderate increases in blood pressure are observed in individuals carrying a variant of the SGK1 gene, affecting as many as 5% of unselected Caucasians. In the same individuals, increased body mass index and a shortening of the QT interval have been observed. The increased body mass index may be partly due to enhanced stimulation of the intestinal glucose transporter SGLT1 and the accelerated cardiac repolarization due to enhanced activation of the cardiac K^+ channel KCNE1.[63] Thus, altered regulation of carriers and channels by SGK1 could account for the coincidence of obesity, hypertension, and altered cardiac action potential.

Paul Welling (University of Maryland, USA): 'Trafficking of K Channels.' The polarized location and cell-surface density of different inwardly rectifying (Kir) channels is precisely controlled in the renal collecting duct for potassium balance. It has previously been shown that Kir 1.1 (ROMK, KCNJ1) (ref. [64]) and Kir 2.3 (refs. [65,66]) channels interact with separate PDZ proteins to control—in different ways—polarized trafficking. In his presentation Welling discussed ongoing efforts to explore the hypothesis that a hierarchical trafficking program controls cell-surface expression of the ROMK channel, involving PDZ-protein interaction and phosphorylation-dependent release from the endoplasmic reticulum. Studies of protein–protein interaction indicate that NHERF-2, a PDZ protein, has the capacity to organize a multimeric protein complex, involving the ROMK channel, protein kinase A, and the aldosterone-induced kinase, SGK-1 (ref. [67]). As determined by *in vivo* and *in vitro* phosphorylation assays, serine 44 in ROMK1 is a substrate for protein kinase A and SGK-1 phosphorylation.[16] Trafficking studies with wild-type phosphorylation mimic (S44D) or phosphorylation null (S44A) Kir1.1a revealed that phosphorylation of serine 44 is required to drive traffic of newly synthesized channels to the plasma membrane. ROMK channels were found to acquire mature glycosylation in a serine 44-phosphorylation-dependent manner, consistent with a phosphorylation-dependent trafficking step within the endoplasmic reticulum/Golgi. Serine 44 is located near a string of three RXR motifs, reminiscent of basic trafficking signals involved in directing early transport steps within the secretory pathway. Mutational analysis revealed that the neighboring arginine residues are necessary for cell-surface expression, identifying a structure that determines export in the biosynthetic pathway. Suppressor mutations in a putative dibasic ER retention signal, located within the cytoplasmic C-terminus (K370A, R371A), restored cell-surface expression and activity of the phospho-null S44A channel to levels exhibited by the phospho-mimic S44D channel.

In summary, Welling has found that phosphorylation of S44 drives an early export step within the secretory pathway by overriding an independent endoplasmic reticulum localization signal. Thus, a balance of intracellular retention and phosphorylation-dependent export controls Kir1.1 cell-surface density. Phosphorylation of S44 by SGK-1 provides a mechanism to explain the requirement of aldosterone for maximal upregulation of the secretory channel observed in dietary potassium loading. Phosphorylation by protein kinase A offers an explanation for vasopressin-dependent regulation of potassium channel density.

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