Regulation of cation transport in the distal nephron by mechanical forces

Lisa M. Satlin, Marcelo D. Carattino, Wen Liu, and Thomas R. Kleyman

1Division of Pediatric Nephrology, Department of Pediatrics, Mount Sinai School of Medicine, New York, New York; and 2Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Submitted 30 May 2006; accepted in final form 12 July 2006

The final regulation of urinary Na\(^+\) and K\(^+\) excretion in the mammalian kidney is accomplished in distal nephron segments including the late distal convoluted tubule, connecting tubule (CNT), and collecting duct (22, 31, 32, 34, 47, 72, 74, 104, 105). Although the late distal tubule and CNT are now considered to be the primary K\(^+\) secretory sites (reviewed in Ref. 79), these segments are difficult to study functionally (e.g., by in vitro micropertusion) due to their short length or highly branched nature. In contrast, the more easily studied straight and unbranched cortical collecting duct (CCD) has led us and others to use this segment as a model of a K\(^+\) secretory epithelium. The CCD, like the CNT, is a heterogeneous structure, composed of two morphologically and functionally distinct cell types. Principal cells absorb Na\(^+\) and secrete K\(^+\) (59, 103, 121). Intercalated cells are specialized for acid/base transport, mediated by a polarized H-ATPase and a Cl/HCO\(_3\) exchanger, but may also contribute to K\(^+\) reabsorption via an apical H-K-ATPase under certain conditions (15, 72, 106, 114). Intercalated cells may also participate in K\(^+\) secretion via maxi-K channels (83, 136, 138). Increases in tubular flow rates enhance both Na\(^+\) reabsorption as well as K\(^+\) secretion in the distal nephron. Mechanisms by which these processes are activated are addressed in this review.

ENA\(_C\) are Mechanosensitive Channels

Cells are potentially exposed to a variety of mechanical forces including indentations, circumferential stretch, high-frequency vibrations, osmotic pressure gradients, hydrostatic pressure, and fluid shear stress (37). In the kidney, frictional forces of the ultrafiltrate flowing through tubules regulate the vectorial transport of glucose (30), chloride (139), magnesium (139), K\(^+\) (32, 53, 73, 136), Na\(^+\) (22, 54, 62, 73, 108), the organization of the cytoskeleton, the synthesis of matrix proteins, and the activity of specific transcription factors (24).

Mechanosensitive ion channels are transducers that respond to mechanical stimuli by changing the permeability of the membrane to specific ions (37). Specific members of the degenerin/epithelial Na\(^+\) channel (DEG/ENaC) family of ion channels are activated by mechanical stimuli. For example, genetic analyses of touch-insensitive Caenorhabditis elegans permitted the development of a model of the touch-transducing complex in which MEC-4 and MEC-10 constitute the core of a mechanosensory transduction complex (23, 75). The subunits that form the conduction pores of DEG/ENaC family members share a similar structure, with two membrane spanning domains connected by a large extracellular loop. The extracellular domains of the ion channel subunits within the C. elegans touch-transducing complex are thought to be associated with a specialized extracellular matrix called the mantle, and tethering of the channel within its extracellular domains allows for mechanical forces to be transmitted to the channel’s gating machinery (21, 23, 75). An extracellular regulatory domain that affects mechanosensitive gating has been identified in the extracellular loop between two cysteine-rich domains (6, 29, 45). The introduction of amino acid residues with bulky side chains at a key site within the region preceding the second membrane-spanning domain (pre-M2) of MEC-4 and MEC-10, termed the degenerin site, generates hyperactive channels that lead to enhanced rates of Na\(^+\) reabsorption as well as K\(^+\) secretion in response to increases tubular flow rates are more complex. Increases in tubular flow rates directly enhance the activity of apical membrane Na\(^+\) channels and indirectly activate a class of K\(^+\) channels, referred to as maxi-K, that are functionally inactive under low flow states. This review addresses the role of biomechanical forces, generated by variations in urinary flow rate and tubular fluid volume, in the regulation of transepithelial Na\(^+\) and K\(^+\) transport in the distal nephron. The question of why the distal nephron has evolved to include a component of flow-dependent K\(^+\) secretion is also addressed.

epithelial Na\(^+\) channel; ROMK; cortical collecting duct; shear stress; SK channel

Address for reprint requests and other correspondence: T. Kleyman, Renal-Electrolyte Div., Univ. of Pittsburgh, A919 Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15261 (e-mail: kleyman@pitt.edu).

http://www.ajprenal.org

0363-6127/06 $8.00 Copyright © 2006 the American Physiological Society
that ENaC is a mechanosensitive channel (2, 48, 108). Initial studies examining this hypothesis produced conflicting results. Awayda et al. (2) showed that in vitro translated bovine α-ENaC, reconstituted in lipid bilayers, was activated by membrane distension produced by a hydrostatic pressure gradient. α-Subunits expressed in cells that lack endogenous stretch-activated cation channels were reported to by activated by pressure applied within a patch pipette (55). Later studies showed that rat αβγ-ENaC was mechanosensitive when reconstituted in lipid bilayers (48). However, conflicting results were reported when oocytes expressing rat ENaC were subjected to an osmotic stimulus (3, 49). When channels were examined in principal cells in mammalian CCDs using a cell-attached patch configuration, application of negative pressure led to an increase in single-channel activity in <30% of patches (90). A lack of reproducibility of stretch-mediated ENaC activation may reflect stretch-induced ATP release from cells that inhibits ENaC to a variable degree by activating purinergic receptors or adenosine receptors (71, 128, 137). It may also reflect differences in the type of mechanical force that was used in these studies.

Recent studies have demonstrated that ENaCs expressed in *Xenopus laevis* oocytes respond to laminar shear stress with an increase in whole cell Na⁺ currents (12, 13). A number of observations suggest that laminar shear stress activates ENaC by increasing channel open probability, Na⁺ channels with a high intrinsic open probability due to a mutation (B5518K) or covalent modification of an introduced Cys residue (αS580C) in the pre-M2 region of the α-subunit were not activated by laminar shear stress, indicating that the response to shear stress reflects an increase in channel open probability (12). Furthermore, Cys substitutions at specific sites in a key region immediately preceding the selectivity filter and second membrane-spanning domain of the α-subunit altered the channel’s response to shear stress, suggesting that this region undergoes conformational rearrangements during channel activation (13). ENaC activation by laminar shear stress might be mediated by a mechanism similar to that for activation of MEC channels in *C. elegans* by mechanical forces. MECs are tethered to extracellular proteins, and this tethering may have an important role in mechanosensing (21). At present, it is unclear whether ENaC subunits are tethered to extracellular proteins, as none have been identified. ENaC is associated with elements of the cytoskeleton (ankyrin, α-spectrin, and actin) (76, 100, 119, 145). An α-spectrin binding site has been identified within the COOH terminus of the α-subunit (100). In addition, short actin filaments alter the behavior of ENaC in bilayers. This functional interaction requires a COOH-terminal domain within the α-subunit (5, 16, 52, 76). ENaC activation by shear stress might not require interactions with the cytoskeleton, as ENaCs with deletions of the COOH-terminal domains of the three subunits are still activated by laminar shear stress when expressed in *X. laevis* oocytes (12).

ENaCs expressed in distal nephron segments are subjected to physiological variations in tubular flow rate. Increasing the rate of perfusion of isolated rabbit CCDs within a physiologically relevant range increased net Na⁺ reabsorption via an amiloride-sensitive pathway, suggesting that ENaCs within the CCD are activated in response to increases in tubular flow rates (22, 73, 108, 136, 138). In vivo micropuncture studies also suggest that Na⁺ reabsorption in the distal nephron is flow dependent. Administration of progressively larger intravenous saline loads, which increased late distal tubular fluid flow rates, increased both distal tubular Na⁺ reabsorption and K⁺ secretion (53, 62). It has been suggested that the delivery of increased amounts of Na⁺ to the distal tubule accounts for the observed flow-dependent increase in the rates of Na⁺ reabsorption (53). However, increases in distal tubular delivery of fluids by infusion of a hypertonic mannitol (15%) solution significantly augment rates of distal Na⁺ reabsorption (62), supporting the flow-dependent nature of Na⁺ reabsorption in the distal segment of the nephron. Flow-dependent increases in tubular Na⁺ reabsorption occur in tubules pretreated with BAPTA to chelate intracellular Ca²⁺, as well as in tubules pretreated with either colchicine or brefeldin A to disrupt apical membrane trafficking (80). Trypsin activates ENaC by increasing channel open probability (10, 14, 117). While trypsin activates Na⁺ reabsorption in tubules perfused at a slow flow rate, it does not further enhance Na⁺ reabsorption in tubules perfused at high flow rates (80). Taken together, these results support observations in oocytes suggesting that shear stress activates ENaCs in CCDs by increasing channel open probability.

**Maxi-K Channels Mediate Flow-Activated K⁺ Secretion in the Distal Nephron**

K⁺ secretion in the distal nephron is dependent on ENaC-mediated Na⁺ absorption (31, 56). Na⁺ passively diffuses from the urine into the CNT/CCD principal cell across the apical membrane via ENaC and is extruded at the basolateral membrane in exchange for the uptake of K⁺ by the Na-K-ATPase. The high cell K⁺ concentration and lumen-negative voltage within this segment, generated by apical Na⁺ entry and its electrogenic basolateral extrusion, create an electrochemical gradient that favors the passive diffusion of cell K⁺ into the luminal fluid through apical K⁺-selective channels (31). High tubular flow rates stimulate net K⁺ secretion in the CNT/CCD and urinary K⁺ excretion (22, 34, 54, 74, 104). This response reflects, at least in part, an increase in delivery to and reabsorption of Na⁺ by principal cells (54, 73, 74, 108), which, in turn, increases the driving force for passive K⁺ efflux across the apical membrane. As discussed above, increases in tubular flow rates activate ENaC, due to an increase in channel open probability.

A flow-induced increase in Na⁺ absorption in the CCD might be expected to increase the lumen negative potential. However, studies by our group (108) and others (22) have shown that an increase in luminal flow rate in the range of 1–15 nl/min increases Na⁺ reabsorption in the microperfused CCD without changing the transepithelial membrane potential (Vₑₑ). The absence of flow-induced change in Vₑₑ, even under conditions where apical K⁺ secretion is inhibited, may reflect an increase in the paracellular permeability to Cl⁻, leading to movement of negative charge out of the lumen (108).

Renal K⁺ secretion was thought to be mediated primarily by the apical K⁺-selective channel ROMK (39). However, loss-of-function mutations in ROMK result in Bartter’s syndrome and are associated with a renal K⁺-wasting phenotype, suggesting that other K⁺ channels have an important role in mediating flow-dependent K⁺ secretion (69, 118). Patch-clamp studies have revealed two apical K⁺-selective channels in the...
mammalian CNT/CCD. The prevalence of a low-conductance channel (42 pS at 37°C) with a high open probability at resting membrane potentials (26, 28, 31, 89, 133) led to the premise that this channel mediates K⁺ secretion under baseline conditions. Heterologous expression of ROMK, originally cloned from rat outer medulla, generates a channel with biophysical and regulatory properties similar to those of the primary K⁺ secretory channel characterized at a single-channel level within CCDs (7, 41, 144).

Less readily detected in cell-attached patches of the apical membrane of the distal nephron is the maxi-K channel. This high-conductance Ca²⁺- and stretch-activated channel is characterized by a conductance of >100 pS, a low density and low open probability at resting membrane potential (26, 27, 87, 107, 111, 127). Apical maxi-K channels are activated by membrane depolarization, elevation of intracellular Ca²⁺ concentration ([Ca²⁺]i), hypotonic stress, and/or membrane stretch (27, 40, 46, 64, 87, 124, 125, 127), and can be selectively blocked by the scorpion venom toxin iberiotoxin (11, 127). The activation profile of the maxi-K channel led us to speculate that these channels mediate flow-stimulated K⁺ secretion. Indeed, an increase in luminal flow rate in the isolated perfused CCD is associated with circumferential stretch, an increase in [Ca²⁺]i, and an increase in ENaC-mediated Na⁺ absorption, potentially depolarizing the apical membrane (108, 137, 138). The observation that flow-stimulated, but not baseline net K⁺ secretion in the CCD is blocked by luminal iberiotoxin, a specific inhibitor of maxi-K but not ROMK channels, supported the notion that maxi-K channels are recruited under certain conditions to secrete K⁺ into the tubular fluid (138). This mechanism of flow-dependent K⁺ secretion mediated by maxi-K channels has been retained in vertebrate evolution. Maxi-K-dependent K⁺ secretion is a phenomenon that has been observed in distal nephron segments of the salamander (Ambystoma tigrinum) (124). The importance of maxi-K channels in mediating K⁺ secretion has been supported by recent studies in mice lacking ROMK. These mice do not have hyperkalemia and secrete K⁺ by a process that is, in part, iberiotoxin sensitive and likely mediated by maxi-K (4, 68, 69).

**Biology of Maxi-K Channels**

Maxi-K channels are generally composed of two subunits (1, 20, 57), a pore-forming α-subunit, encoded by slo, and a regulatory β-subunit. Whereas both mouse and human slo homologs generate maxi-K channels when expressed in X. laevis oocytes (i.e., they are sensitive to voltage and Ca²⁺ and have large single-channel conductances) (8, 19, 77, 88, 102, 130), the β-subunit does not carry current when expressed alone. Coexpression of α- and β-subunits alters the Ca²⁺, voltage, and inhibitor sensitivity of the channel (19, 78, 141). Ca²⁺ binding by maxi-K channels is essential for its physiological activity as Ca²⁺ shifts the voltage-dependent gating of the channel to allow activation to occur with membrane potentials within the physiological range.

The diverse properties of maxi-K channels result in large part from extensive alternative splicing of the α-subunit (8, 19, 77, 88, 102, 130). Heterologous expression of the unique variants revealed differences in their voltage, Ca²⁺, and hormonal sensitivity (99, 102, 130, 143) and, as suggested in more recent studies, their subcellular localization and association with interacting proteins (132). Modifications in channel Ca²⁺ sensitivity are generally associated with alternative splicing in the COOH terminus, which contains the “regulator of conductance of K⁺,” or RCK domains (50, 51, 112). In addition, alternative splicing of the NH₂ terminus occurs near the binding site of the accessory β-subunit of the mammalian homologs.

Two transcripts of the α-subunit have been cloned from rabbit kidney (81). Rbslo1 and 2 are expressed in glomeruli, thin and thick limbs of Henle, and cortical and medullary collecting ducts but are rarely detected in the proximal tubule (81). When expressed in oocytes or Chinese hamster ovary cells, these channels exhibit a high Ca²⁺ and voltage sensitivity (38). Four maxi-K channel β-subunits have been identified at the mRNA level in mammalian kidney (91, 131, 134). A role for the β1-subunit in flow-stimulated urinary K⁺ secretion was recently implicated by the finding that the fractional K⁺ excretion in maxi-K β1 null mice subjected to acute volume expansion was lower than that measured in wild-type animals (92). These data suggest that β1 expression in the CNT may facilitate maxi-K functional expression. Although this observation was consistent with a low K⁺ secretory capacity of the CCD, renal β₁ expression may be limited to the CNT (80, 93).

Flow-stimulated K⁺ secretion is first observed in rabbit CCDs 5 wk following parturition and correlates with expression of maxi-K α-subunit mRNA expression and localization of immunodetectable protein in CCDs (138). Chronic dietary K⁺ supplementation enhances renal K⁺ secretion (120, 122, 140), due in part to an aldosterone-independent increase in density of ROMK channels in the adult rat (89, 133). Increases in dietary K⁺ intake significantly enhance rates of the flow-dependent component of K⁺ secretion, maxi-K α- and β-subunit message expression in CCDs, and apical membrane expression in intercalated cells within CCDs (83). Furthermore, maxi-K α-subunit mRNA splicing is regulated by extracellular K⁺ concentration, to the extent that the latter alters the cell membrane potential. For example, rat pituitary cells normally include the 59-amino acid STREX exon (which enhances Ca²⁺ sensitivity) in ~20% of their endogenous slo transcripts (142). Following addition of 25 mM KCl to the medium for 6 h to depolarize the plasma membrane, a 50% repression of STREX splicing in channel transcripts occurred. This response is mediated by Ca²⁺/calmodulin kinase (CaMK) IV (142). Whether in vivo K⁺ loading alters the expression of maxi-K channel splice variants in CCDs remains to be determined. The STREX exon has been observed in maxi-K α-subunits cloned rabbit kidney (81). It should be noted that patch-clamp studies suggested that K⁺ loading failed to stimulate maxi-K channel activity in rat CCDs (9, 40). However, maxi-K channels are not readily detected using standard cell-attached patch-clamp techniques, where cells are not subjected to the same hydrodynamic forces (including laminar shear stress) that prevail in CCDs in their native cylindrical geometry (87, 107).

ROMK channels are present solely in principal cells within the CCD. In contrast, maxi-K channels have been detected in both principal and intercalated cells in this segment (83, 87, 107, 138). Maxi-K channels were immu-
nolocalized predominately in α-intercalated cells rabbit CCDs, in agreement with patch-clamp studies suggesting that maxi-K α-subunits are expressed primarily in intercalated cells in CCDs (83, 87, 107, 138). Maxi-K channels were localized in principal/CNT cells in CNTs (93). While it is likely that principal/CNT cells in the CNT mediate flow-stimulated K⁺ secretion, the cell type mediating flow-dependent K⁺ secretion in CCDs remains to be determined. The paucity of immunostaining of maxi-K α-subunits in principal cells in CCDs and prevalence of immunodetectable apical protein in α-intercalated cells suggest that the latter cell population may have an important role in flow-dependent renal K⁺ secretion. This possibility raises the question as to the nature of the driving force for K⁺ secretion across the apical membrane of intercalated cells. Although expression of basolateral Na-K-ATPase is less in α-intercalated than in principal cells (101), intracellular K⁺ concentration does not differ between these two cell types (110). A lumen negative potential may be established by adjacent principal cells and transmitted to intercalated cells by “intraepithelial current flow” (58), demonstrated by the observation that inhibition of electrogenic Na⁺ transport by amiloride in principal cells hyperpolarizes the voltage across the apical membrane of adjacent intercalated cells in microperfused rabbit CCDs. Intercalated and principal cells do not appear to be electrically coupled, as demonstrated electrophysiologically (58) and by the absence of an intercellular spread of lucifer yellow injected into unique cell types (137). However, the electrical coupling of ENaC-mediated Na⁺ absorption and H⁺ secretion (35, 60, 63) suggests that electrical coupling may also occur between Na⁺ absorption in principal cells and K⁺ secretion in α-intercalated cells. Muto et al. (82) did not observe intercalated cell Ba²⁺-sensitive K⁺ conductance under high flow states, based on electrical measurements following microelectrode impalement of intercalated and principal cells. However, the intercalated cell subtype that was impaled was not defined. This study was performed in rabbit CCDs, where the β cell is the major intercalated cell type. If the authors were primarily impaling β-intercalated cell, the authors would have not detected flow-dependent K⁺ secretion, which may be limited to α-intercalated cells. Clearly, additional microelectrode studies performed under experimental conditions to elicit flow-stimulated K⁺ secretion by the cells comprising the distal nephron are needed to demonstrate a macroscopic K⁺ conductance in α-intercalated cells.

Sensing Flow in Principal and Intercalated Cells

While ENaC itself may be a mechansensitive channel, there are other mechanosensors within principal and intercalated cells in the distal nephron that respond to changes in tubular flow rates. Acute increases in tubular fluid flow rates lead to increases in [Ca²⁺], in both principal and intercalated cells (67, 137, 138). A number of recent studies have suggested that the central cilium, a structure present in most renal tubular cells except intercalated cells, functions as a flow sensor that transduces an increase in flow to an increase in [Ca²⁺], (66, 84, 94, 95). These structures have been proposed to have roles in the growth and orientation of renal tubular epithelia. Primary cilia have been observed to bend when cultured tubular epithelial cells are subjected to flow (113). Increases in flow rates result in a transient increase in [Ca²⁺], that is blocked by antibodies against polycystin 2 (84).

Intercalated cells, unlike principal cells, are devoid of an apical cilium (25). Mechanisms by which intercalated cells sense flow have not been defined. Principal and intercalated cells do not appear to be coupled by gap junctions in the native epithelium, as they maintain different resting pH, and fail to exhibit intercellular spread of lucifer yellow either under resting conditions or after ATP stimulation (67, 137). Flow over the microvilli and microciniae that decorate the apical surfaces of intercalated cells (25) has been proposed to generate a bending moment equivalent to that created by flow-induced bending of the apical cilium of principal cells. These flow-induced deformations of individual microvilli in the brush border of proximal tubule (36) are predicted to be substantially smaller than the flow-induced bending response of the primary cilium (95, 113). While the bending moment on each microvillus may be very small (~0.01 pN·μm at a flow rate of 30 nl/min), these microvilli are quite numerous (4,000/cell) and collectively generate a combined torque (4 pN·μm) (36) that exceeds that predicted for a central cilium (0.91 pN·μm) in a perfused CCD. Efforts are underway to develop a model for the microvilli/microciniae at the surface of the intercalated cell to provide a theoretical basis for understanding their response to flow.

It should be noted that hydrodynamic drag and torque on the apical cilia and microvilli/microciniae can be increased not only by increasing the velocity of tubular flow but also by increasing the viscosity of the tubular fluid without changing flow rate. Du et al. (17) showed that a approximately fourfold increase in luminal viscosity generated by addition of dextran-80 to the tubule perfusate increased the rate of Na⁺ absorption in proximal tubules microperfused at rates of >5 nl/min. However, a similar increase in luminal viscosity did not alter the rate of net Na⁺ absorption in CCDs perfused at a slow flow rate of 1.5 nl·min⁻¹·mm⁻¹ (108). The apparent discrepancy between the results of the two studies likely reflects the fact that there exists a threshold “shear” necessary to elicit a response in the CCD (67). Specifically, a superfuse flow rate over split-open CCDs of at least 15 ml/s is necessary to generate a [Ca²⁺] response in CCD principal and intercalated cells. If the critical shear threshold is not met, a response would not be observed.

The luminal surface of the distal nephron has a glycoalx composed of glycosaminoglycans as well as other proteins that are set in a hydrated polysaccharide gel (43, 44, 61, 65, 96, 97, 109, 126). Glycosaminoglycans consist of negatively charged disaccharide repeats that are linked to core proteins. These structures have an important role in branching of the ureteric bud (123). Weinbaum and co-workers (129, 135) have proposed that proteoglycans, which have extended “bush-like” structures, are likely to serve as mechanosensors that respond to shear stress. They demonstrated that partial removal of heparan sulfate proteoglycans with heparinase dampened the response of endothelial cells to shear stress (129). The question of whether the luminal glycoalx functions as a flow sensor in CCDs remains to be determined.

There are other mechanisms by which intercalated and principal cells “sense” and respond to flow. In addition to their
localization at the central cilium, polycystins 1 and 2 are expressed at sites of focal adhesions (86). Flow-induced stress that is transmitted to sites of focal adhesions might activate polycystin 2 at these sites. Increases in flow may be associated with increases in tubular volume that activate paracrine/autocrine signaling mechanisms via stretch-induced release of extracellular factors, such as Ca$^{2+}$ or ATP, with concomitant stimulation of Ca$^{2+}$-sensing receptors or purinergic receptors, respectively, on neighboring cells (42, 85). ATP functions as an extracellular signaling molecule through activation of members of the P2X and P2Y receptor families. P2X receptors are Ca$^{2+}$-permeable, nonselective cation channels identified, at the mRNA level, in both principal and intercalated cells (115, 116). Binding of ATP to G protein-coupled P2 purinergic receptors activates PLC, leading to hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate and release of internal Ca$^{2+}$ stores (18, 98). We have identified functional P2Y2 but not P2X receptors on the apical surfaces of both principal and intercalated cells of the CCD (137). However, [Ca$^{2+}$]$_i$ transients induced by an acute increase in tubular fluid flow in the CCD appeared not to be mediated by apical P2 purinergic receptor signaling (137). Pharmacologic blockade of apical P2 receptors by suramin or scavenging of luminal ATP by hexokinase failed to inhibit the flow-stimulated Ca$^{2+}$ response in intercalated cells. The role of nucleotide release in the tubular response to changes in flow rates remains to be fully explored, although extracellular ATP is predicted to inhibit ENaC (70, 71).

Why Does the Distal Nephron Exhibit Flow-Dependent Increases in Na$^+$ Absorption and K$^+$ Secretion?

The clinical relevance of biomechanical regulation of renal tubular Na$^+$ and K$^+$ transport in the distal nephron is readily apparent, particularly in an era where loop and thiazide diuretics are widely used. In contrast, the biological relevance of this phenomenon is less clear. Why did this phenomenon evolve? Present-day humans evolved from vertebrates whose food intake was, at times, likely to include ingestion of K$^+$-rich food on a sporadic basis. Sudden large increases in extracellular K$^+$ concentration are not well tolerated. Mechanisms have evolved to buffer the extracellular K$^+$ concentration, including a shift of K$^+$ from the extracellular to intracellular space. We hypothesize that increases in GFR, associated with a protein-rich meal, lead to an increase in distal flow rates that will activate a number of events within the distal nephron, as summarized in Fig. 1. These events include 1) an activation of Na$^+$ reabsorption, 2) an increase in [Ca$^{2+}$]$_i$, and 3) an activation of maxi-K channels. The flow-dependent activation of ENaCs and maxi-K channels will enhance renal K$^+$ excretion, which provides an additional mechanism to buffer extracellular K$^+$.

Future Directions

Many questions remain to be addressed regarding mechanisms by which ion channels in the distal nephron are regulated by flow. Specific questions include 1) do intercalated cells, which have traditionally not been considered to participate in K$^+$ secretion, secrete K$^+$ in response to flow; 2) does ATP release modulate the effects of laminar shear stress on ENaC activation, and if so, is this response mediated by ligand binding to apical or basolateral purinergic receptors; 3) how do ENaCs sense flow; 4) do ENaCs need to be tethered to extracellular proteins to exhibit flow dependent activation, and what are the extracellular proteins that tether ENaC; 5) what is the molecular identity of luminal Ca$^{2+}$ entry pathways that are activated in response to flow and how are they regulated; 6) what are the maxi-K splice variants and $\beta$-subunit isoforms expressed in the distal nephron, are they cell specific, and are they altered in development and by epigenetic factors; 7) does flow-stimulated K$^+$ secretion reflect enhanced trafficking of preformed channels to the apical membrane (increase in number) as well as a change in channel open probability; 8) what is the role of specific kinases, such as PKC and CaMK, in the activation of maxi-K channels in response to flow; 9) do changes in extracellular volume status (and circulating vasoressin and aldosterone levels) affect maxi-K expression or localization; and 10) does flow affect intercalated cell-mediated acid-base transport in the CCD?

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-038470 and DK-051391. W. Liu was supported by a Polycystic Kidney Disease Foundation Fellowship Grant.
REFERENCES


