An unexpected journey: conceptual evolution of mechanoregulated potassium transport in the distal nephron

Rolando Carrisoza-Gaytan,1 Marcelo D. Carattino,2 Thomas R. Kleyman,2 and Lisa M. Satlin1

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

Carrisoza-Gaytan R, Carattino MD, Kleyman TR, Satlin LM. An unexpected journey: conceptual evolution of mechanoregulated potassium transport in the distal nephron. Am J Physiol Cell Physiol 310: C243–C259, 2016; doi:10.1152/ajpcell.00328.2015.—Flow-induced K secretion (FIKS) in the aldosterone-sensitive distal nephron (ASDN) is mediated by large-conductance, Ca2+/stretch-activated BK channels composed of pore-forming α-subunits (BKα) and accessory β-subunits. This channel also plays a critical role in the renal adaptation to dietary K loading. Within the ASDN, the cortical collecting duct (CCD) is a major site for the final renal regulation of K homeostasis. Principal cells in the ASDN possess a single apical cilium whereas the surfaces of adjacent intercalated cells, devoid of cilia, are decorated with abundant microvilli and microplicae. Increases in tubular (urinary) flow rate, induced by volume expansion, diuretics, or a high K diet, subject CCD cells to hydrodynamic forces (fluid shear stress, circumferential stretch, and drag/torque on apical cilia) that are transduced into increases in principal (PC) and intercalated (IC) cell cytoplasmic Ca2+ concentration that activate apical voltage-, stretch- and Ca2+-activated BK channels, which mediate FIKS. This review summarizes studies by ourselves and others that have led to the evolving picture that the BK channel is localized in a macromolecular complex at the apical membrane, composed of mechanosensitive apical Ca2+ channels and a variety of kinases/phosphatases as well as other signaling molecules anchored to the cytoskeleton, and that an increase in tubular flow rate leads to IC- and PC-specific responses determined, in large part, by the cell-specific composition of the BK channels.

kidney; mechanoregulation; potassium transport; WNK kinases; cilia

THE ALDOSTERONE-SENSITIVE distal nephron (ASDN), which is composed of the distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct (CCD) (9, 95, 115, 122, 133), plays a critical role in the final renal regulation of electrolyte and water homeostasis. Increases in urinary flow rate in the ASDN, induced by volume expansion, administration of diuretics or a high K (HK) diet, subject epithelial cells therein to 1) fluid shear stress (FSS); 2) circumferential stretch acting parallel and perpendicular, respectively, to the tubular wall; and 3) drag/torque on apical cilia of principal cells and microvilli/microplicae of intercalated cells (28, 40, 54, 229). Cumulative evidence from studies performed in isolated CCDs microperfused in vitro indicate that these hydrodynamic forces are transduced into increases in net transepithelial Na absorption through the epithelial Na channel (ENaC) (137, 178), and K secretion, the latter dependent on flow-induced increases in intracellular Ca2+ concentration ([Ca2+]i) that activate apical stretch-, voltage-, and Ca2+-activated K (referred to as BK) channels (110, 236, 237). BK channels are now considered to not only mediate flow-induced K secretion (FIKS), but also play a major role in K adaptation to dietary K loading (10, 110, 112, 113, 140, 157, 158, 168, 174, 236, 237).

Glossary

AII Angiotensin II
AE4 Anion exchanger 4
ASDN Aldosterone-sensitive distal nephron
AT1R Angiotensin type 1 receptor
ATP Adenosine triphosphate
BAPTA 1,2-bis(o-aminophenoxy)ethane-N,N,N’N’-tetraacetic acid
BK High-conductance K [channel]
BP Blood pressure
[Ca2+]i Intracellular Ca2+ concentration
cAMP Cyclic adenosine monophosphate
cGMP Cyclic guanosine monophosphate

Address for reprint requests and other correspondence: L. M. Satlin, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1198, New York, NY 10029 (e-mail: lisa.satlin@mssm.edu).

http://www.ajpcell.org 0363-6143/16 Copyright © 2016 the American Physiological Society
exchange for the uptake of K. K secretion in the ASDN requires excreted by the kidney, with the remaining output, the latter regulated primarily by K secretion into K Secretion in the ASDN

C244 MECHANOREGULATED K TRANSPORT IN DISTAL NEPHRON

Review

Total body K content depends on the balance between intake and output, the latter regulated primarily by K secretion into the urinary (tubular) fluid in the ASDN (49, 62, 64, 65, 86, 122, 124, 173). In the adult, whose homeostatic goal is to maintain a state of zero K balance, ~90% of the K ingested daily is excreted by the kidney, with the remaining ~10% eliminated by the gut. K secretion in the ASDN requires an apical permeability to K and a2) a favorable electrochemical gradient, which is determined by apical Na entry through ENaC and its electrogenic Na-K-ATPase-mediated basolateral extrusion in exchange for the uptake of K.

In animals fed a normal K diet, net K secretion in the microperfused CCD is completely dependent on ENaC-mediated Na absorption (137, 197, 198). However, in rats fed a HK diet for as little as 18 h, a fraction of distal K secretion appears to occur via an amiloride-insensitive and thus ENaC-independent pathway (59). Indeed, basolateral Na uptake via the Na/H exchanger can sustain basolateral pump activity in principal cells and net K secretion in the isolated perfused rabbit CCD in the absence of significant Na absorption (139). Note that the paracellular permeability to Na and K is low in this segment under physiologic conditions (147).

In the fully differentiated ASDN, high tubular flow rates stimulate K secretion and urinary K excretion (49, 65, 91, 123, 173). This response is due, at least in part, to increased delivery to and reabsorption of Na via ENaC (91, 123, 137, 178), which in turn increases the driving force for passive K efflux across the apical membrane. In addition, multiple lines of evidence suggest that ENaC is a mechanoregulated channel that is directly activated by increases in FSS (4, 137, 178), which would help maintain the electrochemical driving force for K secretion under high flow states.

K secretory channels. The high-conductance BK channel (also known as maxi-K, Slo1, or KCNMA1) was the first ion channel described, in 1984, at the single-channel level in the kidney by the patch-clamp technique (85). The pore-forming α-subunit of this channel was originally cloned from the slowpoke locus of Drosophila (slo) and is encoded by the KCNMA1 gene in humans (1, 6, 25, 213). The BK channel is activated by membrane depolarization, elevation of [Ca2+]i, hypoosmotic stress, and/or membrane stretch (57, 58, 79, 85, 90, 101, 105, 150, 153, 177, 181, 199–201, 208, 209). However, its vanishingly low open probability (Po) at the resting membrane potential and [Ca2+]i in the ASDN (57, 58, 60, 150, 177, 181, 222) called into question its physiologic relevance to urinary K secretion, and it thus received little subsequent attention over the subsequent 15 years. In contrast, the high prevalence and Po of the apical ATP-sensitive small K conductance (SK) channel, first characterized in 1989, made this the likely candidate for the primary K secretory channel in the ASDN (57, 60, 66, 176, 222, 245). The SK channel is encoded by ROMK (Ksα1.1) (20, 80, 257) and, like the BK channel, has been detected in both rat and rabbit CNT/CCD.

After its initial identification, the SK/ROMK channel, considered to be the primary K secretory channel in the ASDN, was the focus of numerous studies aimed at examining its role in the renal regulation of K excretion. However, cumulative evidence from studies of ontogeny and disease over the past two decades suggested that other K channels contribute to K secretion under conditions where SK/ROMK channel-mediated K secretion was limited. The first evidence that at least two K channels mediate physiologically relevant K secretion was from studies performed to examine the molecular basis for the observations that, in contrast to the adult, growing infants and children 1) must maintain a state of positive K balance, increasing their total body K from ~8 meq/cm body height at birth to >14 meq/cm body height by 18 years of age (26, 56), and 2) have a limited capacity for urinary K excretion (44, 205). Indeed, the notion that the growing subject is a “sink” for K was elegantly demonstrated in a study in which plasma K concentrations were compared in newborn piglets administered K loads (25 meq·kg−1·day−1) in either water or milk for the...
first 40 h of life. Whereas K-loaded animals provided water alone lost weight and experienced life-threatening hyperkalemia and paralysis, piglets fed the equivalent amount of K in milk grew well and remained normokalemic (127).

To address the question as to how the newborn retains K, as is necessary for growth, CCDs isolated from maturing rabbits were microperfused in vitro and the rates of net transepithelial K secretion were measured at slow (∼1) and fast (∼5 nl·min⁻¹·mm⁻¹) luminal flow rates. Net K secretion was absent at 2 wk of age, but the rate of K secretion in CCDs from weanling (4-wk-old) rabbits, perfused at slow flow rates, was ∼75% of that measured in adult (>6-wk-old) animals perfused at slow flow rates (173) (Fig. 1). Yet, FIKS, robust in adult animals, was absent in weanling tubules (173). Thus, there appeared to be a temporal dissociation between basal K secretion and FIKS. The absence of K secretion early in life was not due to a limited capacity of the CCD for Na absorption (identical to that in adult rabbits at all flow rates; Fig. 1) or basolateral pump activity (38, 173, 177), but instead, was due to the absence of an apical membrane permeability to K (176). Subsequent studies in maturing rabbits revealed that the postnatal appearance of conducting SK/ROMK channels and apical immunodetectable BKα subunit protein in the rabbit CCD coincided with the appearance of basal K transport at 3 wk (before weaning) and FIKS at 5 wk (postweaning) postnatal age, respectively (173, 237).

The second observation that questioned the primacy of the SK/ROMK channel as the K secretory channel in the CCD was that infants with antenatal Bartter syndrome due to loss-of-function mutations in ROMK (type II Bartter syndrome) are hyperkalemic soon after birth, but develop modest hypokalemia beyond the neonatal period (53, 170, 190). Hyperkalemia is not persistent, as would be expected in the absence of the presumed primary K secretory channel. An initial clue to the identity of this “other” channel was provided by the finding that the kaliuresis observed in adult ROMK knockout mice (116, 117) was sensitive to iberiotoxin (IbTX) (10), a selective blocker of the BK channel whose receptor resides in the pore of the α-subunit (10, 27, 61, 208); IbTX does not inhibit SK/ROMK (10) or Ca²⁺-activated SK3 channels (18). This observation provided compelling evidence that the high distal flow rates characteristic of this murine model of Bartter syndrome, due to an impaired urinary concentrating ability, activate BK channels and thus urinary K excretion.

The repertoire of K secretory channels identified in the ASDN has continued to expand over the past decade, although there appears to be significant species- and cell-specificity in their functional expression. Most recently, a small-conductance voltage-insensitive K channel, SK3 (Kc8.2.3), activated by a TRPV4-mediated increase in [Ca²⁺], and inhibited by apamin (136), has been functionally identified in mouse collecting duct where it localizes to the luminal border of both principal and intercalated cells (18). Yet another apical K channel, Kv1.3, present in rat intercalated cells, can be recruited to secrete K into the tubular fluid in response to dietary K loading (29).

**Molecular biology of the BK channel.** Functional mammalian BK channels are composed of four pore-forming α-subunits, alone or assembled together with regulatory β-subunits (6, 47, 94, 118, 131) that modulate the Ca²⁺ affinity, voltage sensitivity, and pharmacology of the channel as well as its association with interacting proteins (25, 76, 130, 213, 221). Whereas both mouse and human slo homologues generate BK channels when expressed in *Xenopus laevis* oocytes, i.e., they are generally sensitive to voltage and Ca²⁺ and have large single-channel conductances (25, 46, 125, 171, 213), the β-subunit does not carry current when expressed alone. Ca²⁺ binding is essential for physiological BK channel activity as Ca²⁺ shifts the depolarization required for channel opening to allow activation to occur within the physiological range of membrane potentials.

Alternative splicing of slo in the COOH terminus generates variants that differ in their responses to changes in Ca²⁺ and voltage, regulation by protein phosphorylation, palmitoylation, and other signaling cascades (31, 172, 186, 187, 211, 213, 243, 247, 255, 258), as well as trafficking and cell localization (92, 98, 120, 221, 251). Among the five variants of the mouse BKα subunit COOH terminus that have been identified, three are expressed at significant levels in kidney (% of total renal BK channel mRNA levels) (31): ZERO, resulting from splicing of exon 19 to 23 (75%); e21, resulting in insertion of a 59-amino acid cysteine-rich stress-axis regulated exon (STREX) between exons 19 and 23 (10%); and Δe23, resulting from the skipping of exon 23, thereby splicing exon 19 to 24, which leads to a frameshift that introduces a premature stop codon within exon 24 (5%). The STREX variant demonstrates a left shift in the Ca²⁺ sensitivity of the channel compared with the ZERO variant and slower rates of deactivation (31, 172). Δe23 is not functionally expressed at the cell surface and acts as a dominant negative in terms of cell surface expression by trapping other BK channel splice variant α-subunits in the endoplasmic reticulum and perinuclear compartments (31, 98).

---

**Fig. 1.** Flow stimulation of net K secretion (left) and Na absorption (right) in the maturing rabbit CCD. Net transport was measured at slow (∼1 nl·min⁻¹·mm⁻¹), moderate (∼2 nl·min⁻¹·mm⁻¹), and fast (∼5 nl·min⁻¹·mm⁻¹) flow rates in tubules isolated from 2-, 4-, and 6-wk-old rabbits (n = 5–6 per age group) and microperfused in vitro. Net K secretion was absent at 2 wk of age and could not be stimulated by an increase in tubular fluid flow rate. Although a 5-fold increase in flow rate stimulated net Na absorption at 4 wk to levels comparable to those observed at 6 wk of age, no flow-induced increase in net K secretion (FIKS) was detected at 1 mo of life in response to an increase in flow rate to 5 nl·min⁻¹·mm⁻¹. Weaning occurs in the rabbit by 4 wk of age. FIKS was clearly evident by 6 wk of age. [Adapted from Satlin (173) and Woda et al. (237).]
Four BK channel β-subunits have been identified at the mRNA and/or protein level in mammalian kidney and appear to be differentially expressed along the nephron (67, 140, 156, 214, 228). The observation, from studies in heterologous systems, that coexpression of β1 with BKα increases the Ca$^{2+}$ sensitivity and charybdoxin binding affinity of the channel (46, 130) suggests that this is the logical subunit to comprise the CCD BK channel, which exhibits robust IbTX-sensitive FIKS. However, the BKβ1-subunit (as well as the β2-subunit; see below) contains an endocytic sorting signal that promotes endocytosis of BKα and thus a reduction in surface expression of the channel (212, 252). Clearance studies in mice with targeted deletion of BKβ1 reveal attenuated FIKS (157, 158). BKβ1 KO mice are also hypertensive due to hyperaldosteronism secondary to adrenal hypersensitivity to the elevated plasma K concentration that accompanies the reduction in renal BKα/β2-mediated K secretion (67, 68). While these data provide solid support for a role of the BKα/β1 channel in mediating FIKS (157, 158), immunodetectable β1 is not expressed in the rabbit midcortical CCD (50, 140), a segment that consistently exhibits robust IbTX-sensitive FIKS (110, 112, 236). However, BKα/β1 has been identified in rabbit in a cortical segment that is most likely initial CCD (156–158). Immunodetectable BKα localizes to the apical membrane of mouse CNT cells along with β1 (156, 158), mouse CCD intercalated cells without β1 (67, 68, 158), and is also found at the apical surface of intercalated cells in rabbit CCD (50, 140).

Coexpression of β2 (214) or β3 (24, 214, 242) subunits with the BKα-subunit generally results in complete or partial, respectively, inactivation of channel currents. The inhibitory nature of the β2- and β3-subunits suggests they are unlikely candidate subunits for the BK conductance in the native mammalian CCD. Subunit β2 increases the sensitivity for voltage at Ca$^{2+}$ concentrations of greater than 1.5 μM, alters the gating behavior of the expressed channels in a Ca$^{2+}$-dependent manner and, if glycosylated, dramatically reduces IbTX association rates, thereby rendering the channel relatively insensitive to this toxin (24, 88, 132, 219). Immunodetectable BKα/β3 is found in the TALH, DCT, and intercalated cells (but not CNT/principal cells) of the mouse distal CNT and CCD (67). Mice lacking BKβ4 have no detectable phenotype under baseline conditions when fed a normal diet (68).

The composition of the native channel in the CCD, whether composed of an α-subunit alone, an α-subunit associated with a β1-subunit, a nonglycosylated (IbTX-sensitive) β3-subunit, or an as yet undescribed β-subunit, remains to be clarified. Recently, a second family of BK channel auxiliary subunits has been identified. These leucine-rich-repeat containing subunits (LRRCs), also referred to as γ-subunits, allow BK channels to open at near-physiological Ca$^{2+}$ concentration and membrane voltage in nonexcitable cells (246, 254). It is as yet uncertain as to whether this subunit is expressed in the ASDN.

The BK channel may associate with other auxiliary proteins in a macromolecular complex. Both Slo (Slowpoke-binding protein) and Slo1 (Slo-interacting protein 1) interact with the cytoplasmic tail domain of Slo to regulate channel activity by directing the localization and distribution of channels within cells (182, 241). Activity of the BK channel is also modulated (reduced) by the 14-3-3 protein, acting through Slo and signaling proteins (192, 259).

Genetic ablation studies in mice have more clearly defined the roles of the BK vs. SK/ROMK channels in K homeostasis. Targeted deletion of BKα (168), β1 (156–158), or β4 (177, 225) subunits leads to marked attenuation of the kaliuresis induced by high-flow conditions in vivo, including, respectively, pharmacologic V2 receptor blockade, volume expansion, or HK diet. These observations provide solid support for the notion that the SK/ROMK channel mediates constitutive K secretion in animals ingesting a normal K diet whereas the BK channel mediates FIKS (110, 112, 113, 236). The BK channel also plays a critical role in the adaptation to a dietary K load (105, 140) and maintenance of circulating volume and blood pressure (BP) by regulating Na and fluid homeostasis (68, 69, 157). Mice with genetic ablation of BKα present primarily with a neurological phenotype but also exhibit hypertension, proposed to be secondary to BK channel deficiency in vascular smooth muscle (180).

Cell specificity of K channel expression. Principal cells, the majority cell type in the ASDN, mediate Na absorption via ENaC and K secretion via the SK/ROMK channel. These cells possess a single apical cilium that projects into the lumen (51, 225). This organelle bends in response to fluid shear (183) and transduces mechanical perturbation into increases in [Ca$^{2+}$], (160–162). Intercalated cells secrete H$^+$ via an apical vacuolar H$^+$-ATPase (α-cells) or HCO$_3^-$ via apical pendrin (β-cells) but can also, under certain conditions, reabsorb K via an apical H$^+$-K$^+$-ATPase (37, 119, 175, 184, 189). Non-A non-B intercalated cells possess both apical H$^+$-ATPase and pendrin (48, 93). β-Intercalated cells can also absorb NaCl via a thiazide-sensitive apical Na-dependent Cl/HCO$_3^-$ exchanger (NDCBE) that operates in tandem with pendrin (103). Intercalated cells lack a central cilium, at least in rabbit, but are decorated with a plethora of apical microvilli and microplecia (51). Whereas ENaC and SK/ROMK channels are restricted to principal cells in rat and rabbit ASDN, BK channels are detected by patch-clamp analysis in both principal and intercalated cells in these species (105, 150, 153, 177). However, the incidence of conducting BK channels in intercalated cells exceeds that in principal cells (105, 150, 153). Emerging evidence further suggests that the activation profiles of BK channels are cell specific and may differ between species. For example, the intercalated cell BK channel in rabbit, but not rat, has been reported to be activated by stretch, independent of Ca$^{2+}$, as evidenced by patch-clamp studies performed after chelation of free Ca$^{2+}$ with EGTA in the pipette or the bath solutions (150).

The identity of the specific cell(s) in the ASDN responsible for FIKS remains uncertain. Principal cells possess robust basolateral Na-K-ATPase activity and are considered to mediate transepithelial Na absorption via ENaC and K secretion via ROMK, as discussed above (57, 60, 176, 222). However, the density of conducting and immunoreactive apical BK channels is low in these cells (50, 68, 105, 140, 153, 158, 177, 215, 237). In fact, it is difficult to reconcile the relative absence of immunodetectable apical BKα in principal cells of rabbit (50, 140, 237) and mouse (67, 68, 158) CCD with the electrophysiologic evidence for conducting channels therein (105, 150). While we initially proposed that BKα splice variants in principal cells were not recognized by the chicken anti-BKα antibody we raised against an epitope at the extreme COOH terminus of the protein (237), similar results were reported using a mouse anti-Slo1 antibody (StressMarq) raised against a
highly conserved upstream (a.a. 690–715) epitope (135). Using an in vitro immunoperfusion approach with 3D reconstructions of confocal images, we have recently localized immunodetectable BKα to the principal cell central cilium, a structure that has been difficult to detect by conventional analysis of kidney sections (unpublished observations).

In support of a role for principal cells in BK channel-mediated K secretion are the observations that BK channel activity in CCD principal cells increases in HK-fed rats (105) and in rabbits and rats fed a normal K diet, following inhibition of PKA (112) or p38/ERK (105). These data suggest that a pool of quiescent (constitutively suppressed) BK channels in principal cells can be activated and recruited to secrete K under high flow conditions or in response to dietary K. Furthermore, FIKS in mouse kidney (10) and rabbit CCD (33, 111, 112, 237) is inhibited by IbTX. Coassembly of BKα with β1-subunits, which have been identified in an intracellular compartment and at the apical membrane of principal cells in rabbit initial CCD (156, 158), generates a channel sensitive to 50 nM IbTX whereas the β2-subunit, present in intercalated cells (but not principal cells) of the mouse distalCNT and CCD (67), renders BKα-subunits resistant to low nanomolar concentrations of IbTX if it is glycosylated (132, 144). While mice lacking BKβ4 have no detectable phenotype under baseline conditions when fed a normal diet (82), BKβ4 KO mice subject to volume depletion exhibit an attenuated kaliuretic response (157, 158). These data are consistent with the notion that FIKS is mediated by a BKα or BKα/β1 channel.

Support for a role for intercalated cells in FIKS includes the findings that the density of immunodetectable apical BKα-subunits in rabbit (50, 140) and mouse (67, 68, 158), and functional (105, 150, 153, 177) BK channel activity are greater in intercalated than in principal cells. Furthermore, apical BKα-subunit expression in intercalated cells is increased in response to a HK diet (140). In addition, the apical membrane voltage of the intercalated cell is depolarized relative to that of the principal cell (14, 96, 153), predicting that BK channel Po would be high (due to the voltage sensitivity of Po), reducing the requirement for elevation of [Ca2+]i to open the channel (25, 153). Of interest are recent studies (185) that indicate that phosphorylation of the mineralocorticoid receptor (MR) at S843, which prevents ligand binding and activation of the MR, is found exclusively in intercalated cells in kidney. Hyperkalemia increases whereas angiotensin II (AII) via the AT1R and WNK4 (both increased in volume depletion) decrease MR S843-P levels. The observation that dephosphorylation of MR S843-P, which lies downstream of AII and WNK4 signaling, inhibits K secretion implicates selective MR inactivation in intercalated cells in the adaptation to hyperkalemia. Finally, WNK4 inhibits BKα functional and protein expression in heterologous expression systems (see below) (223, 250, 260).

Sustained luminal K secretion by intercalated cells would require a robust basolateral K uptake pathway, such as that provided by the Na-K-ATPase, to maintain a high steady-state [K]i. Immunocytochemical studies in rat kidney, performed using antibodies directed against the Na-K pump α-subunit and an antigen retrieval technique, reveal modest labeling, at best, of intercalated cells in the CCD (171). Yet, Na-K-ATPase pump activity (13) and ouabain-sensitive currents are not detected in these cells whereas pump activity is readily detected in principal cells in rat CCDs (153). Although basolateral Na-K-ATPase antigenicity has been identified in rabbit intercalated cells (167), electron microprobe analysis of intracellular electrolyte concentrations in individual cells in isolated perfused rabbit CCDs revealed a significantly smaller increase in [Na+]i in intercalated vs. principal cells exposed to basolateral ouabain (179). In sum, these immunocytochemical and functional analyses suggest that intercalated cells may have insufficient Na-K-ATPase activity to sustain high rates of luminal K secretion. Evidence now suggests that, as in the colon, K can be taken up at the basolateral membrane of the CCD not only by the pump but also by the Na-K-2Cl cotransporter NKCC1 (19). In fact, we reported that BK channel-mediated FIKS is dependent on a basolateral bumetanide-sensitive Cl-dependent transport pathway, proposed to be NKCC1 based on its immunolocalization in both intercalated and principal cells in the CCD (111). The findings that l/j mice with genetic disruption of NKCC1 exhibit higher serum K concentrations with appropriately low urinary K excretion compared with wild-type mice (134, 218) and 2, in human subjects, the 24-h kaliuresis that follows once-daily administration of furosemide is lower than following administration of an equinatriuretic dose of a thiazide diuretic (165, 166) are consistent with an important role of NKCC1 in distal K secretion. Still uncertain is how Na+, taken up at the basolateral membrane by NKCC1, is extruded back out of intercalated cells. An intriguing possibility, yet to be tested, is that Na is extruded at the basolateral membrane via anion exchanger 4-mediated Na-HCO3 cotransport; using cotransporter-specific antibodies, AE4 labeling has been identified along the basolateral membrane of mouse and rabbit β-ICs (30).

Regulation of BK Channel Activity By Cell Signaling Components

An increase in luminal flow rate in the microperfused rabbit CCD is transduced into a biphasic increase in [Ca2+]i, composed of an initial rapid transient spike in [Ca2+]i to >300 nM, reflecting IP3-mediated release of Ca2+ from internal stores, followed by a plateau elevation (150 nM) sustained for at least 20 min of high flow due to luminal Ca2+ entry into cells, presumably through store-operated and/or TRP channels (110, 113). These flow-induced changes in [Ca2+]i are apparent in both principal and intercalated cells in the CCD. Luminal IbTX inhibits the plateau elevation of [Ca2+]i, but not the initial [Ca2+]i spike (110, 113), [Ca2+]i exceeding 10 μM is generally required to activate BK channels in neurons at membrane potentials of 0 mV (24). However, elevations of [Ca2+]i to this magnitude are expected to be cytotoxic and thus are likely elicited only in “Ca2+-signaling domains,” macromolecular complexes in the plasma membrane that include Ca2+ channels, that allow for temporal and spatial restriction of the Ca2+ signal (7, 16, 143). In the rat distal nephron, elevation of [Ca2+]i to the 200–500 nM range is adequate to stimulate BK channels in intercalated cells studied at a zero membrane potential (153).

FIKS, a “read-out” of BK channel activity, is critically dependent on a flow-induced increase in [Ca2+]i, in the rabbit CCD. Indeed, FIKS is absent in microperfused CCDs in which the cytosolic Ca2+ signal has been eliminated by removal of luminal Ca2+, chelation of [Ca2+], with BAPTA, inhibition of IP3-stimulated Ca2+ release from the endoplasmic reticulum.
(ER) by 2-aminoethyl diphenyl borate, or thapsigargin-induced depletion of ER Ca\(^{2+}\), treatments that do not affect flow-stimulated Na absorption (137). Among the well-described cellular processes activated by an increase in [Ca\(^{2+}\)]\(\text{i}\), is the stimulation of exocytosis in various secretory cells (21). Pretreatment of CCDs with colchicine to disrupt microtubule function or brefeldin-A to inhibit delivery of preformed channels from the intracellular pool to the plasma membrane inhibits FIKS (but not flow-stimulated Na absorption) (137), underscoring the critical importance of exocytosis in BK channel-mediated FIKS.

An unanswered question is how a transient increase in [Ca\(^{2+}\)]\(\text{i}\), elicited by an acute increase in luminal flow rate leads to sustained FIKS. The persistent activation of channel-mediated ion currents in response to a transient stimulus has, in other systems, been attributed to posttranslational modifications such as phosphorylation or dephosphorylation, multimerization, and the activities of cyclases, esterases, and proteases (22). In fact, the COOH terminus of BK\(\alpha\) contains multiple kinase and phosphatase motifs that associate with partners to regulate channel gating and signaling pathways. Among these effectors are cAMP-dependent PKA (104, 163, 188, 211, 258), PKC (11, 72, 73, 164, 188, 220, 240, 258), cGMP-dependent PKG (3, 11, 196, 258), and cSrc (2, 109). The BK leucine zipper region serves as an anchor for a PKA-associated regulatory complex (118).

We and others have begun to examine whether sustained activation of the BK channel in response to a transient stimulus is due to direct phosphorylation or dephosphorylation of the channel itself or an associated accessory or regulatory protein. The results of these studies, summarized below, indicate that the BK channel in principal cells of the CCD, in the absence of luminal flow or perfused at slow luminal flow rates, is tonically inhibited by both PKA (112) and MAPK (105). These observations suggest that hydrodynamic forces generated by increases in tubular fluid flow activate/inhibit these kinase-associated cell-specific signaling pathways that in turn influence apical BK channel function. In fact, numerous studies, primarily in endothelial cells, have shown that FSS and circumferential stretch regulate multiple signaling molecules that can initiate and propagate mechanical signals through a network of pathways (reviewed in refs. 35, 41, 230).

**PKA and PKC.** The \(\alpha\)-subunit of the reconstituted BK channel is phosphorylated by PKA and PKC (104). The BK channel in the native CCD is also regulated by these kinases. Specifically, luminal perfusion of CCDs with myristoylated PKI (mPKI), a peptide inhibitor of the free catalytic subunit of PKA, increased net K secretion in rabbit CCDs perfused at a slow flow rate (112). The sensitivity of this enhanced K flux to IbTX implicated the BK channel as a target for regulation by PKA and suggested that the apical channel is constitutively inhibited by this kinase. However, patch-clamp analysis of rat CCD indicated that mPKI activated (demonstrated by an increase in \(N_p\)) BK channels solely in principal cells (112). In fact, mPKI inhibited \(N_p\), of BK channels in intercalated cells. Luminal calphostin C, which binds to the diacylglycerol (DAG) binding site, or C1 domain, in PKC and other proteins, like mPKI, increased net IbTX-sensitive K secretion in CCDs perfused at a slow flow rate. However, the target of calphostin C inhibition appeared not to be PKC as luminal bisindolylmaleimide and Gö6976, inhibitors of classical Ca\(^{2+}\)-dependent (PKC-\(\alpha\), PKC-\(\beta\)) and novel Ca\(^{2+}\)-independent (PKC-\(\delta\) and PKC-\(\epsilon\)) PKC isoforms, failed to enhance net K secretion at the slow flow rate (112). To the extent that the calphostin C target has not yet been identified, the remainder of this discussion will focus on PKA. Flow stimulation of net Na absorption was detected in all CCDs treated with these inhibitors.

The transport and patch-clamp results summarized above led us to conclude that the apical BK channel in the principal cell is tonically inhibited by PKA under low flow conditions. The observation that inhibition of “apical” PKA and an increase in luminal flow rate lead to comparable increases in IbTX-sensitive net K secretion suggests that flow may reduce cAMP available to activate PKA, thereby releasing the BK channel or a closely associated regulatory protein from tonic inhibition in a signaling complex at the apical membrane, as has been described for the channel in smooth muscle and brain (reviewed in ref. 118). Of note is that rat cholangiocyte cilia respond to an increase in luminal flow with a TRPP1/2 channel complex-mediated increase in [Ca\(^{2+}\)]\(\text{i}\) that activates ciliary adenylate cyclase 6, which in turn suppresses local and global cAMP signaling (126). This cascade, if triggered by flow in native CCDs, would be expected to release the BK channel from constitutive inhibition and uncover BK channel-mediated K secretion in ciliated principal cells, but not intercalated cells, devoid of cilia (112).

The variability in functional response of the BK channels to distinct kinases (and phosphatases) has been proposed to be due to alternative splicing of the \(\alpha\)-subunit (99, 211, 258), association of \(\alpha\)-subunits with distinct regulatory \(\beta\)-subunits (46, 130) and/or associated proteins (118, 182, 241), as well as through differential assembly of BK channels with protein kinase/phosphatase signaling complexes (118, 220). In fact, we have proposed that the cell specificity of the mPKI effect in the CCD may reflect the presence of unique BK\(\alpha\) splice variants in principal cells that are inhibited (e.g., STREX, which introduces a new PKA consensus site into the channel sequence) vs. activated (e.g., ZERO, e20, e22) by PKA (31). PKA phosphorylation of the conserved COOH-terminal PKA consensus site (RQPPS\(_{99}\)) in all four \(\alpha\)-subunits is required for channel activation, whereas phosphorylation of only a single STREX insert at its PKA consensus motif inhibits the channel (118, 210, 211). The cell specificity of regulation by PKA may also reflect differential expression of BK\(\beta\)-subunits as it is assumed that the \(\beta\)-subunits in principal (\(\beta_1\)) and intercalated (\(\beta_3\)) cells differ. In support of the latter possibility is the observation that endogenous PKA activates channel activity in HEK293 cells heterologously expressing the human BK\(\alpha\)-subunit but decreases channel activity when the \(\alpha\)- and \(\beta_1\)-subunits are coexpressed (46). Note that the precise composition of BK channels in principal and intercalated cells has yet to be defined.

**MAPK.** BK channel activity, measured as \(N_p\) by patch-clamp analysis, is constitutively inhibited by p38 and ERK MAPK in rat principal but not intercalated cells (105). Specifically, single-channel recordings of principal cells in split-open CCDs from rats fed a normal K diet showed that p38 blockade with the specific inhibitor SB202190 dramatically increased the number and \(P_o\) of apical BK channels. PD098059, a drug that blocks ERK activation, had no significant effect alone but had an additive effect on channel activity when applied with the p38 inhibitor (105). The cell specificity of the MAPK.
response, as for the PKA effects, is proposed to be due to variability in BK channel composition between the two cell types. Phosphorylation of ERK and p38 is stimulated by WNK4, which inhibits BK channel activity (250). A HK intake suppresses the phosphorylation of p38 and ERK (105), which is expected to increase BK channel activity in the CCD, enhancing BK channel-mediated net K secretion.

The patch-clamp results summarized above predict that, if principal cells are responsible for BK channel-mediated K secretion, treatment of CCDs microperfused at slow flow rates with a p38 inhibitor should increase net K secretion. However, this is not observed (unpublished observations); indeed, the rates of net K secretion and Na absorption in CCDs perfused and bathed with SB203580, an inhibitor of p38 MAPK catalytic activity that binds to the ATP binding pocket but does not inhibit phosphorylation of p38 MAPK by upstream kinases, were identical. How do we reconcile the difference between the patch-clamp data and the perfusion data? The simplest explanation is that BK channels in intercalated but not principal cells in the native CCD secrete K. Assignment of a role for intercalated cells in mediating FIKS would also resolve the conundrum raised by the finding that monolayers of mpkCCD principal-like cells, subjected to 0.4 dyn/cm² of FSS, a force considered to be comparable to that experienced by an ex vivo CCD microperfused at a physiologically fast flow rate, express a ~2-fold greater abundance of p-ERK and p-p38 compared with cells not exposed to FSS (28). FSS-induced activation of these MAPK elements would be expected to inhibit BK channel activity and FIKS if mediated by principal cells but would have no effect on BK channel-mediated K secretion if affected by intercalated cells. That differential expression of p38 and p-ERK expression in mpkCCD cells compared with unstretched controls (28).

WNKs. WNK (with-no-lysine) kinases play important roles in the regulation of Na absorption and K secretion in the ASDN, especially in response to changes in dietary K intake (102, 231). Mutations in full-length kinase-active WNK1 (L-WNK1) or WNK4, both expressed in the ASDN, cause familial hyperkalemic hypertension (FHHt; also referred to as pseudo-hypokalemia type II), a hereditary disease characterized by hypertension, hyperkalemia, and hyperchloremic metabolic acidosis with normal or slightly elevated aldosterone levels (155, 232). L-WNK1 and WNK4 kinases regulate Na transporters in the ASDN, including NCC and ENaC (78, 83, 89, 100, 169, 203, 232, 244, 248). L-WNK1 stimulates ENaC via activation of serum and glucocorticoid-inducible kinase 1 (sgk1) (244), whereas WNK4, expressed in principal cells and intercalated cells of C57/B6 mice (185), inhibits ENaC by stimulating clathrin-dependent endocytosis (89, 169). It is well established that L-WNK1 and WNK4 inhibit ROMK expression by enhancing channel endocytosis (39, 77, 89, 102, 169, 216). FHHt-causing WNK4 mutations enhance the inhibition of ROMK and are associated with a further reduction in ROMK surface density and K secretion beyond that mediated by wild-type WNK4 (89).

Mechanisms by which WNK1 regulates transporters are complex, as an alternatively spliced WNK1 isoform lacking the NH₂ terminus and kinase domain and functioning in a dominant interfering mode is specifically expressed in the kidney (kidney-specific WNK1, or KS-WNK1) (43, 149). KS-WNK1 antagonizes the effects of L-WNK1 and WNK4 on ROMK endocytosis (102, 204, 216). A high ratio of L-WNK1 to KS-WNK1 in the CCD enhances ENaC-mediated Na reabsorption and limits ROMK-mediated K secretion, resulting in dissociation of the two cation fluxes thought to be tightly coupled to the basolateral Na-K-ATPase. Acute (102, 216) and chronic (148) dietary K loading of the rat and mouse, respectively, increases the expression of KS-WNK1 at the message and protein level in whole kidney, although increases in KS-WNK1 mRNA expression appear to be restricted to the TALH and DCT and not CCD (34).

In contrast to the hyperkalemia observed in FHHt, loss-of-function ROMK mutations in Bartter syndrome are associated with hypokalemia (190). The kaliuresis observed in this setting is mediated by BK channels (10) and highlights the importance of this channel in renal K secretion. The effects of WNK kinases on BK channel activity are now being elucidated. WNK4 reduces whole cell and surface expression of BKα in HEK293 cells by a process that is dependent on its kinase activity, sensitive to inhibitors of lysosomal degradation, and also dependent on MAPKs. We also found that WNK4 enhances ubiquitination of BKα, consistent with a role for WNK4 in targeting the channel for internalization and/or degradation via an ubiquitin-dependent pathway in these cells (223). These results are consistent with the notion that WNK4 enhances the routing of BK channels to lysosomes in a ubiquitin-dependent manner (260) that is also dependent on activation of MAPKs (250). In contrast to its effect on ROMK, L-WNK1 significantly increases BKα whole cell and functional expression in HEK293 cells (114, 224). L-WNK1 expression reduced ERK phosphorylation, whereas knockdown of WNK1 increased the ubiquitination of BKα, effects that are opposite to the effects seen with WNK4 (114). Surprisingly, both KS-WNK1 and kinase dead L-WNK1 increased BKα whole cell expression in HEK293 cells, suggesting that WNK1 kinase activity is not required to enhance BKα expression (224).

KS-WNK1 KO mice exhibit an increase in BKα expression (2-fold in α and β1 and 50% decrease in β3) in kidney (71), consistent with a role of L-WNK1 in increasing BK channel expression. As BK channels are expressed at multiple sites in the nephron, it will be important to define whether there are changes in BK channel expression in specific cell types in the KS-WNK1 KO mice. It was also reported that loss-of-function mutations in KS-WNK1 lead to a reduced capacity of the CCD for basal K secretion but not FIKS in microperfused CCDs (33). These findings are consistent with a role for L-WNK1 in selectively inhibiting ROMK channels in the ASDN, while not inhibiting BK channels (33).

Although it is known that L-WNK1 and WNK4 show distinct subcellular distribution patterns (232), emerging evidence has revealed cell type-specific expression of WNK kinases. WNK-1 expression in the CCD is low in rabbits fed a LK diet but is selectively upregulated in β intercalated cells in HK-fed animals, where it is localized to the apical membrane and subapical region of this specific cell population (224). This finding has important implications regarding the adaptive response to K loading, where urinary flow rates are increased (12, 23, 52, 193, 215). The selective upregulation of L-WNK1 in β intercalated cells would promote BK channel-mediated K secretion to maintain K balance. A lack of upregulation of
L-WNK1 in principal cells would prevent L-WNK1-dependent inhibition of ROMK expression.

If L-WNK1 levels are increased in some individuals with FHHt, why do they present with hyperkalemia? L-WNK1-dependent downregulation of ROMK expression should reduce K secretion by principal cells. While L-WNK1 should increase BK channel expression in β intercalated cells, reduced distal Na delivery and tubular flow due to enhanced NaCl absorption in the early DCT should blunt BK channel-mediated K secretion in latter aspects of the ASDN. Furthermore, thiazide diuretics correct hyperkalemia in patients with FHHt, consistent with the notion that BK channel-mediated FIKS can be observed in FHHt if distal flow rates are increased.

Regulation of BK Channel Function By Extrinsic Factors

Flow rate. Apical BK channels, normally quiescent at slow urinary flow rates, are activated in the ASDN at high flows by mechanoinduced increases in [Ca\textsuperscript{2+}], as described above. Primary cilia in principal cells function as flow sensors, whose mechanical deflection by urinary flow leads to Ca\textsuperscript{2+} entry into cilia through cilia TRPP2 channels, likely associated with TRPV4 as heteromeric channels, with downstream effects including a global increase in cytoplasmic [Ca\textsuperscript{2+}], (17, 36, 45, 63, 74, 97, 110, 142, 145, 146, 161, 162, 239). However, initial studies in MDCK cells, later extended to microperfused tubules, suggest that flow-induced cytosolic [Ca\textsuperscript{2+}] transients are not due exclusively to direct Ca\textsuperscript{2+} entry through ciliary TRP channels into the cell body but involve release of autocrine/paracrine factors such as ATP (87, 191) and PGE\textsubscript{2} (55) that activate purinergic (81, 84, 87, 125, 159) and EP (55) receptor-associated signaling cascades. Indeed, the primary cilia is functionally distinct from the cytoplasm and represents only a tiny fraction of the total cell volume (45, 202). Mechanoactivation of Ca\textsuperscript{2+} channels in cilia would be expected to provide only a small “rivulet” of Ca\textsuperscript{2+} at its base into the large cytoplasmic volume, without significant perturbation of global [Ca\textsuperscript{2+}], (45). However, the apical cilium has been proposed to be essential for ATP secretion and serve as a chemosensor for secreted ATP; an increase in [Ca\textsuperscript{2+}] presumably primes “releasable” pools of ATP beneath the cell surface for secretion in response to chemical, osmotic, and mechanical stimuli which, in turn, activates autocrine/paracrine purinergic signaling, transduced by nearby receptors (84). Note that the ATP-permeable hemichannel connexin 30, localized to the apical membrane of β intercalated cells, is an important route for release of ATP into the urine, particularly in response to changes in Na balance (129, 191). Pannexin-1 (Panx1) channels, expressed along the apical membranes of intercalated cells, may also participate in ATP secretion as Panx1-deficient mice excrete less ATP than do wild-type animals (75). Luminal flow stimulates PGE\textsubscript{2} release in the CCD, which inhibits Na absorption and enhances FIKS. As expected, indomethacin enhances flow-stimulated Na absorption but dampens FIKS (55). Studies by Gueutin et al. (70) have implicated β intercalated cells as critical for ATP release, which, via purinergic P2Y2 receptor activation, leads to production and release of PGE2 in the CCD.

Dietary K. Chronic dietary K supplementation enhances renal K secretion (195, 238), due to aldosterone-induced increase in driving force favoring K secretion in the ASDN, as well as aldosterone-independent mechanisms. Aldosterone rapidly induces sgk1 in the ASDN (32) that, in turn, stimulates Na reabsorption, in part by inhibiting retrieval of ENaCs from the luminal membrane (42). Aldosterone also rapidly induces expression of GILZ in mpkCCD cells that stimulates ENaC-mediated Na transport by inhibiting ERK signaling (194).

A HK diet is associated with increases in apical membrane expression and activity of both BK and SK/ROMK channels (105, 140, 154, 222). Expression of ROMK but not BK channels may be enhanced by increases in circulating levels of aldosterone (50, 217, 249). An increase in dietary K intake for as little as 6 h increases SK/ROMK channel density in rat CCD, an adaptation well described after 10–14 d of HK intake (152, 222). While this effect appears to require an increase in plasma K, the observation that adrenalectomized rats fail to exhibit an increase in SK channel density in response to HK intake suggests that circulating steroid levels play a permissive role in this process, at least in this species (152). In contrast, microperfused CCDs isolated from K adapted rabbits exhibit enhanced K secretion even after adrenalectomy (235). Furthermore, the apical K conductance of the CCD is increased in both control and adrenalectomized rabbits fed a HK diet (138). These data suggest that aldosterone may be necessary for K adaptation in rat, but not necessarily in rabbit.

Chronic (~10 day) dietary K loading enhances FIKS in the rabbit CCD (140), a functional adaptation accompanied by changes in BK-α and β-subunit mRNA expression, as well as BK-α localization in intercalated cells (140). Specifically, steady-state abundance of mRNA encoding BK-α and β\textsubscript{2,4} subunits in single CCDs from HK fed animals exceeds that detected in control K-fed (CK) rabbit. Immunofluorescence microscopy revealed a predominantly intracellular distribution of BK-α in kidneys from animals fed a 5K diet whereas robust apical labeling was detected in α intercalated cells in HK kidneys, consistent with redistribution from an intracellular pool to the plasma membrane.

Dietary K loading (10% K) increases IbTX-sensitive K secretion in in vivo microperfused mouse distal nephron (10), consistent with the finding by others (168) that a 5% HK diet for 6 days increases expression of BK channel protein in kidney. Mice with genetic ablation of BK\textsubscript{β1} exhibit, at baseline, reduced urinary K and Na clearances, conditions that are exacerbated when the animals are fed a HK (5% K) diet (69). The effect of dietary K loading on BK channel activity in rat appears to be inconsistent. Whereas some patch-clamp studies have found that K loading fails to stimulate BK channel
activity in rat CCD (79, 153, 222), others report that the probability of detecting apical BK channels and channel activity in the CCD is greater in rats fed a HK diet compared with a standard K diet (105). These discrepancies may be related to the use, in some studies, of a high KCl (vs. NaCl with only 5 mM K) bath solution that depolarizes the cell membrane potential and thereby inactivates BK channels (105). Additionally, as channel activation appears to require an increase in \[\text{Ca}^{2+}\], to at least 200 nM (153), channel activity may not be readily detected by standard cell-attached patch-clamp analysis in which the channels within the pipette are protected from hydrodynamic forces expected to generate a localized increase in \[\text{Ca}^{2+}\] (113).

The signals responsible for upregulation of BK channel activity in response to dietary K loading remain to be fully identified. BK channels in smooth muscle and endothelial cells are activated by epoxyeicosatrienoic acid (EET), a product of CYP-epoxygenase dependent arachidonic acid metabolism (5, 15). CYP-epoxygenases, including CYP2C23, are expressed in the CNT and CCD (121, 141, 206) and are upregulated in response to a HK diet, which also leads to an increase in 11,12-EET expression in isolated CCDs (207). Inhibition of CYP-epoxygenase in isolated micropерfused rabbit CCDs with \(\text{M}-\text{methylsulfonyl-6-(propargyloxyphe-}
\text{nyl)hexanamide (MS-PP0H)}\) abolished IbTX-sensitive and thus BK channel-mediated FIKS but not ROMK-mediated baseline net K secretion (207). These data suggest that a CYP-epoxygenase-dependent arachidonic acid pathway, stimulated in the kidney in response to dietary K loading, contributes to BK channel-mediated K secretion in the CCD.

HK diets increase tubular fluid flow rate in the ASDN in vivo via a reduction in fluid reabsorption in the proximal tubule (23) and NCC activity in the DCT (12, 52, 193, 215). High tubular flow rates will increase \([\text{Ca}^{2+}]\) (see above), which, in turn, should activate BK channels directly but may activate the phosphatase calcineurin, which has been reported to inactivate p38 MAPK (106).

Chronic dietary K restriction leads to a reduction in renal K secretion due to inhibition of K secretion by ROMK and BK channels (105, 140, 227) and enhanced H-K-ATPase mediated K absorption in the CCD (189, 233, 234). Administration of a HK diet to rabbit for 10 days eliminates FIKS in the CCD, a functional adaptation accompanied by suppression of steady-state abundance of mRNA encoding BK\(\alpha\)- and \(\beta_{2_a}\)-subunits in single CCDs isolated from these animals (140).

Protein tyrosine kinase (PTK)-mediated phosphorylation of ROMK enhances endocytosis of the channels in the rat CCD under conditions of dietary K restriction (107, 108) via a dynamic process involving clathrin-coated pits (253). Tyrosine phosphorylation of ROMK channels decreases with a HK diet, and it increases in the face of dietary K restriction (227). K restriction suppresses the expression of protein phosphatase 2B (PP2B) catalytic subunits but increases expression of PP2B regulatory subunit in both rat and mouse kidney, without an effect on expression of PP1 and PP2A. Inhibition of PP2B decreases ROMK channel activity through stimulation of p38 and ERK in the CCD (256). Activation of PTK and MAPKs reduces activity (and vice versa) of not only ROMK, but also BK channel activity in mouse and rat CCD (8, 105, 226). The increase in BK channel activity detected after 7 days of a HK diet is associated with a reduction in the phosphorylation of p38 and ERK (105).

**Emerging Picture of Mechanoregulation of K Secretion in the ASDN**

Cumulative evidence garnered over the past 15 years reveals that the BK channel in the ASDN mediates FIKS and is thus likely the channel responsible for the kaliuresis associated with administration of diuretics or induced by volume expansion. Furthermore, this channel plays a major role in the renal adaptation to dietary K loading. The data presented in this review, reported by ourselves and others, provide compelling evidence that the BK channel in the ASDN is localized in a macromolecular complex at the apical membrane, composed of mechanosensitive apical \(\text{Ca}^{2+}\) channels and a variety of kinases/phosphatases as well as other signaling molecules (Fig. 2). This channel is present in both principal cells, traditionally considered to mediate Na absorption and K secretion, and intercalated cells, long considered to effect acid-base transport not K secretion. However, the composition of the BK channel appears to be cell-specific as is the regulation of channel activity by endogenous effectors. In principal cells, BK channels, likely to be a BKα- STREX/β1 composition, are constitutively inhibited by PKA and MAPK elements (Fig. 2). Physiologic stimuli, such as an increase in urinary flow rate or dietary K loading, may be able to activate these silent pools of apical BK channels by suppressing endogenous inhibitors. The intercalated cell BK channel, likely to be composed of α-subunits and possibly β3 or another accessory subunit, is activated by PKA and not affected by phosphorylation of p38 and ERK MAPK (Fig. 2). Emerging evidence suggests that BK channels are regulated by WNKS, but the effects of the distinct kinases in this family may be cell type-specific. The discrete cell type mediating FIKS in the ASDN, especially after dietary K loading, remains to be precisely identified, as do the specific mechanical forces and mechanoinduced signaling cascades activated by an increase in luminal fluid flow.

**REFERENCES**


**AUTHOR CONTRIBUTIONS**


**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).


136. Muto S, Sansom S, Giebisch G.


139. Nikolic G, Osswald H.


207. van der Lubbe N, Moes AD, Rosenbaek LL, Schoep S, Meima ME, Danser AH, Fenton RA, Zietse R, Hoorn EJ. K⁺-induced natriuresis is preserved during Na⁺ depletion and accompanied by inhibition of the...


