Ca\textsuperscript{2+} dependence of flow-stimulated K secretion in the mammalian cortical collecting duct

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Liu W, Morimoto T, Woda C, Kleyman TR, Satlin LM. Ca\textsuperscript{2+} dependence of flow-stimulated K secretion in the mammalian cortical collecting duct. Am J Physiol Renal Physiol 293: F227–F235, 2007. First published March 27, 2007; doi:10.1152/ajprenal.00057.2007.—Apical low-conductance SK and high-conductance Ca\textsuperscript{2+}-activated BK channels are present in distal nephron, including the cortical collecting duct (CCD). Flow-stimulated net K secretion (J\textsubscript{Kc}) in the CCD is J\textsubscript{Kc} blocked by iberiotoxin, an inhibitor of BK but not SK channels, and 2J\textsubscript{Kc} associated with an increase in [Ca\textsuperscript{2+}]\textsubscript{i}, leading us to conclude that BK channels mediate flow-stimulated J\textsubscript{Kc}. To examine the Ca\textsuperscript{2+} dependence and sources of Ca\textsuperscript{2+} contributing to flow-stimulated J\textsubscript{Kc}, J\textsubscript{Kc} and net Na absorption (J\textsubscript{Na}) were measured at slow (~1) and fast (~5 nl min\textsuperscript{-1} mm\textsuperscript{-1}) flow rates in rabbit CCDs microperfused in the absence of luminal Ca\textsuperscript{2+} or after pretreatment with BAPTA-AM to chelate intracellular Ca\textsuperscript{2+}. 2-aminoethoxydiphenyl borate (2-APB), to inhibit the inositol 1,4,5-trisphosphate (IP\textsubscript{3}) receptor or thapsigargin to deplete internal stores. These treatments, which do not affect flow-stimulated J\textsubscript{Kc} (Morimoto et al. Am J Physiol Renal Physiol 291: F663–F669, 2006), inhibited flow-stimulated J\textsubscript{Kc}. Increases in [Ca\textsuperscript{2+}]\textsubscript{i}, stimulated exocytosis. To test whether flow induces exocytic insertion of preformed BK channels into the apical membrane, CCDs were pretreated with 10 \mu M colchicine (COL) to disrupt microtubule function or 5 \mu g/ml brefeldin-A (BFA) to inhibit delivery of channels from the intracellular pool to the plasma membrane. Both agents inhibited flow-stimulated J\textsubscript{Kc} but not J\textsubscript{Na} (Morimoto et al. Am J Physiol Renal Physiol 291: F663–F669, 2006), although COL but not BFA also blocked the flow-induced [Ca\textsuperscript{2+}]\textsubscript{i} transient. We thus speculate that BK channel-mediated, flow-stimulated J\textsubscript{Kc} requires an increase in [Ca\textsuperscript{2+}]\textsubscript{i}, due, in part, to luminal Ca\textsuperscript{2+} entry and ER Ca\textsuperscript{2+} release, microtubule integrity, and exocytic insertion of preformed channels into the apical membrane.

maxi-K channel; BK channel; SK channel; mechanoregulation; ENaC

THE FINAL REGULATION OF URINARY K excretion (and Na absorption) in the mammalian kidney is accomplished in distal nephron segments including the connecting tubule (CNT) and cortical collecting duct (CCD) (19, 24, 32, 44). The magnitude of K secretion in these segments is determined by the electrochemical gradient, generated by apical Na entry through the benzamid-sensitive epithelial Na channel (ENaC) and its electrogenic basolateral extrusion, favoring K diffusion from the cell into the tubular fluid, and the permeability of the apical membrane to K. High tubular flow rates stimulate net K secretion in the distal nephron (19, 25, 32, 44, 71). This response reflects, at least in part, an increase in delivery to and reabsorption of Na by principal cells (25, 35, 48), which in turn increases the driving force for passive K efflux across the apical membrane.

Electrophysiological analyses have identified two types of K channels in apical cell-attached patches of CNT and CCD cells. The prevalence of the low-conductance secretory K (SK) channel and its high P\textsubscript{0} at the resting membrane potential (16, 45, 67) have led to the premise that this channel mediates K secretion under baseline conditions. The ibeirotoxin (IBX)-sensitive high-conductance BK (maxi-K) channel, which is characterized by a low P\textsubscript{0} at the resting membrane potential and low intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) (16, 40, 46, 54), is activated by membrane depolarization, elevation of [Ca\textsuperscript{2+}]\textsubscript{i}, hypotonic stress, and/or membrane stretch (40, 52, 54).

The BK channel exists as a multimeric protein complex composed of two integral membrane subunits (2, 33): a pore-forming \alpha-subunit, encoded by slo, and a regulatory \beta-subunit. Whereas both mouse and human slo homologs generate BK channels when expressed in Xenopus laevis oocytes, i.e., they are sensitive to voltage and Ca\textsuperscript{2+} and have large single-channel conductances (10, 15, 58), the \beta-subunit does not carry current when expressed alone. Ca\textsuperscript{2+} binding by BK channels is essential for physiological activity as Ca\textsuperscript{2+} shifts the voltage-dependent gating of the channels to allow activation to occur within the physiological range of membrane potentials (10).

We previously reported that net flow-stimulated K secretion in the isolated perfused adult rabbit CCD is blocked by IBX (72) and is associated with increases in net Na absorption and [Ca\textsuperscript{2+}]\textsubscript{i} (31, 71, 72). These data and studies by others (3) reporting that mice lacking ROMK secrete K by a process that is, at least in the late distal tubule, IBX sensitive have led to the conclusion that the BK channel mediates flow-stimulated K secretion. However, the precise relationship between the flow-induced increases in [Ca\textsuperscript{2+}]\textsubscript{i} and net Na absorption and stimulation of BK-mediated K secretion is as yet uncertain. The primary purpose of the present study was to test the hypothesis that flow stimulation of net K secretion, mediated by BK channels, is critically dependent on a flow-induced increase in [Ca\textsuperscript{2+}]\textsubscript{i}, associated with luminal Ca\textsuperscript{2+} influx and internal store release, two sources of Ca\textsuperscript{2+} we previously identified as giving rise to the flow-induced [Ca\textsuperscript{2+}]\textsubscript{i} transient in the CCD (31). To the extent that immunodetectable apical BK \alpha-subunits (36, 72) and conducting BK channels detected by patch-clamp analysis (40) predominate in intercalated cells, which do not reabsorb Na but are primarily responsible for H\textsuperscript{+}/HCO\textsubscript{3} transport (47, 50), we also sought to examine the dependence of

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flow-stimulated net K secretion on net Na absorption, a transport process mediated exclusively by principal cells (27, 46).

METHODS

Animals. Adult (>6 wk) female New Zealand White rabbits obtained from Covance (Denver, PA) were housed in the Mount Sinai School of Medicine Center for Comparative Medicine. All animals were allowed free access to water and chow. Animals were euthanized in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Animal protocols were approved by the IACUC Committee at the Mount Sinai School of Medicine.

Microperfusion of isolated rabbit CCDs. Kidneys were removed via a midline incision, and single tubules were dissected freehand in cold (4°C) Ringer solution containing (in mM) 135 NaCl, 2.5 K2HPO4, 2.0 CaCl2, 1.2 MgSO4, 4.0 lactate, 6.0 t-alanine, 5.0 HEPES, and 5.5 D-glucose, pH 7.4, 290 ± 2 mosmol/kgH2O, as previously described (31, 72). A single tubule was studied from each animal.

Isolated collecting ducts were microperfused in vitro as previously described (31, 72). Briefly, each isolated tubule was immediately transferred to a temperature- and O2-CO2-controlled specimen chamber, mounted on concentric glass pipettes, and perfused and bathed at 37°C with Burg’s perfusate containing (in mM) 120 NaCl, 25 NaHCO3, 2.5 K2HPO4, 2.0 CaCl2, 1.2 MgSO4, 4.0 Na lactate, 1.0 Na3 citrate, 6.0 t-alanine, and 5.5 D-glucose, pH 7.4, 290 ± 2 mosmol/kgH2O (31, 72). All tubules, including those in which cation transport was subsequently measured at room temperature, were equilibrated for 45 min at 37°C during which time the perfusion chamber was continuously suffused with a gas mixture of 95% O2-5% CO2 to maintain pH of the Burg’s solution at 7.4. The bathing solution was continuously exchanged at a rate of 10 ml/h using a syringe pump (Razel, Stamford, CT).

Measurement of net cation transport. Transport measurements were performed in the absence of transepithelial osmotic gradients and thus water transport was assumed to be zero. Three to four samples of tubular fluid were collected under water-saturated light and thus water transport was assumed to be zero. Three to four samples of tubular fluid were collected under water-saturated light and thus water transport was assumed to be zero. Three to four samples of tubular fluid were obtained for analysis. The K and/or Na concentrations of perfusate and collected tubular fluid were determined by gelatin disc photometry and the rates of net cation transport (in pmol·min⁻¹·mm⁻¹ tubular length) were calculated using standard flux equations, as previously described (44). The calculated ion fluxes were averaged to obtain a single mean rate of ion transport for the luminal flow-stimulated net K secretion on net Na absorption, a transport process mediated exclusively by principal cells (27, 46).

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RESULTS

Flow-stimulated K secretion requires an increase in Ca2+, due to luminal Ca2+ entry. To examine whether flow stimulation of net K secretion in the CCD is dependent on an increase in Ca2+, CCDs were pretreated with 20 μM BAPTA-AM, a membrane-permeant Ca2+ chelator, and the rates of net K secretion were measured at slow (1.3 ± 0.3 mmol·min⁻¹·mm⁻¹) and fast (5.7 ± 0.3 mmol·min⁻¹·mm⁻¹) flow rates. We have recently reported that pretreatment of CCDs with BAPTA did not inhibit flow-stimulated Na absorption (35). In contrast to the approximately threefold increase in net K secretion (−10.5 ± 2.2 to −27.2 ± 4.0 pmol·min⁻¹·mm⁻¹; n = 7; P < 0.05; Fig. 1) detected in control CCDs subject to a comparable fivefold increase in luminal flow rate from 1.1 ± 0.1 to 5.3 ± 0.3 mmol·min⁻¹·mm⁻¹, chelation of intracellular Ca2+ completely inhibited flow-stimulated but not baseline net K secretion (−6.2 ± 2.4 to −5.5 ± 1.0 pmol·min⁻¹·mm⁻¹; n = 4; P = not significant). Of note was that an acute increase in luminal flow rate led to a significantly blunted increase in
Flow-stimulated K secretion requires an increase in [Ca\textsuperscript{2+}], due to luminal Ca\textsuperscript{2+} entry. Flow-stimulated but not baseline K secretion is IBX sensitive (72). To confirm that this IBX effect is due to inhibition of the BK channel and not the genesis of the increase in [Ca\textsuperscript{2+}], CCDs were pretreated with IBX and the effect of an acute increase in luminal flow rate on [Ca\textsuperscript{2+}], was examined. As shown in Fig. 2C, CCDs (n = 4) pretreated with luminal IBX exhibited a [Ca\textsuperscript{2+}] transient similar to that observed in control tubules (Fig. 2A), indicating that IBX does not directly inhibit the early events leading to an increase in [Ca\textsuperscript{2+}].

To examine whether flow stimulation of net K secretion in the CCD is dependent on internal Ca\textsuperscript{2+} store release, CCDs were pretreated with basolateral 2-APB (10 \textmu M), a cell-permeant inhibitor of the IP\textsubscript{3} receptor, or thapsigargin (100 nM), an irreversible inhibitor of ER Ca\textsuperscript{2+}-ATPase that prevents refilling of intracellular Ca\textsuperscript{2+} pools and leads to depletion of internal stores. We previously reported that pretreatment of CCDs with basolateral thapsigargin or 2-APB completely eliminated the flow-stimulated increase in [Ca\textsuperscript{2+}], (31). Although 2-APB may inhibit store-operated Ca\textsuperscript{2+} channels (20), low concentrations (<20 \textmu M) of this agent preferentially inhibit IP\textsubscript{3} receptors (41). Pretreatment of CCDs with thapsigargin inhibited both baseline and flow-stimulated net K secretion (Fig. 3); an increase in luminal flow from 1.3 ± 0.4 to 5.7 ± 1.2 nl·min\textsuperscript{-1}·mm\textsuperscript{-1} in CCDs pretreated with thapsigargin failed to stimulate net K secretion (−1.1 ± 1.1 to 0.3 ± 2.5 pmol·min\textsuperscript{-1}·mm\textsuperscript{-1}; n = 3; P = not significant). Pretreatment of CCDs with 2-APB also inhibited flow-stimulated but not baseline net K secretion (−7.9 ± 3.0 to −11.9 ± 1.3 pmol·min\textsuperscript{-1}·mm\textsuperscript{-1}; n = 4; P = not significant; Fig. 3) as the luminal flow rate was increased from 0.9 ± 0.1 to 5.7 ± 0.8 nl·min\textsuperscript{-1}·mm\textsuperscript{-1}. In contrast, the flow-stimulated increase in net Na absorption in these same thapsigargin (10.8 ± 3.7 to 66.5 ± 13.9 pmol·min\textsuperscript{-1}·mm\textsuperscript{-1}; n = 3; P < 0.05) and 2-APB (17.0 ± 4.2 to 54.8 ± 7.1 pmol·min\textsuperscript{-1}·mm\textsuperscript{-1}; n = 4; P < 0.05)-treated CCDs was identical to that observed in control tubules (Fig. 3). These data suggest that flow stimulation of net K secretion, but not Na absorption, requires an increase in [Ca\textsuperscript{2+}], due to both luminal Ca\textsuperscript{2+} entry and internal Ca\textsuperscript{2+} release from IP\textsubscript{3}-sensitive stores.

Flow-stimulated K secretion requires microtubule integrity and exocytic trafficking. Intracellular Ca\textsuperscript{2+} regulates exocytosis and secretion in epithelial cells via, at least in part, microtubule-dependent movement of secretory vesicles (1, 8). To examine the role of the microtubules in flow-stimulated K secretion, CCDs were pretreated with colchicine (10 \textmu M), a

![Flow-stimulated K secretion is dependent on an increase in [Ca\textsuperscript{2+}], and luminal Ca\textsuperscript{2+} entry in microperfused cortical collecting ducts (CCDs). Net K secretion was measured at tubular flow rates of ~1 and 5 nl·min\textsuperscript{-1}·mm\textsuperscript{-1} in untreated control CCDs (C, control; n = 7) or tubules pretreated with 20 \textmu M BAPTA-AM, a chelator of intracellular Ca\textsuperscript{2+} (n = 4), or microperfused in the absence of luminal Ca\textsuperscript{2+} (Ca\textsuperscript{2+}-free perfusate ± EGTA; n = 8). Means ± SE. *P < 0.05 vs. transport rate at the same flow rate in control CCDs.](http://ajprenal.physiology.org/)

![Summary of flow-induced [Ca\textsuperscript{2+}] changes in principal (gray) and intercalated (open) cells in perfused CCDs studied at 37°C in the presence of no inhibitors (control; n = 10; A), BAPTA-AM (n = 5; B), or iberiotoxin (IBX; n = 4; C). Baseline resting (BL; at slow flow rates) and flow-induced peak (P) [Ca\textsuperscript{2+}] values are given, as are the values detected at specific times (min) after initiation of high flow and 20 min after the flow rate was reduced (recovery, R). Means ± SE. *P < 0.05 vs. baseline value. #P < 0.05 vs. peak [Ca\textsuperscript{2+}] in controls.](http://ajprenal.physiology.org/)
microtubule inhibitor (36) that also inhibits vesicle transport between the TGN and the plasma membrane of polarized epithelial cells (21, 63). We previously reported that flow stimulation of net Na absorption is not significantly affected by pretreatment with this agent (35). In contrast, an increase in tubular fluid flow rate from 1.0 ± 0.2 to 4.4 ± 0.3 nl·min⁻¹·mm⁻¹ failed to stimulate net K secretion in CCDs pretreated with colchicine (−7.1 ± 2.1 to −6.8 ± 1.8 pmol·min⁻¹·mm⁻¹; n = 6; P = not significant; Fig. 4). CCDs pretreated with lumicolchicine (10 μM), the inactive analog of colchicine, exhibited a typical threefold increase in net K secretion (−8.0 ± 1.8 to −21.0 ± 5.3 pmol·min⁻¹·mm⁻¹; n = 5; P < 0.05) in response to a fivefold increase in luminal flow rate from 1.0 ± 0.1 to 4.7 ± 0.1 nl/min (Fig. 4). Of note was our finding that an acute increase in luminal flow rate led to a significant increase in [Ca²⁺]i in lumicolchicine-treated CCDs (n = 3; Fig. 5A), a response that was blunted in colchicine-treated segments (n = 5; P < 0.05 compared with lumicolchicine-treated CCDs; Fig. 5B). These data are consistent with a requirement of an intact microtubule system and/or exocytic trafficking of preformed BK and/or Ca²⁺ channels to the apical membrane for flow-stimulated net K secretion.

To further explore whether flow stimulation of net K secretion requires exocytic insertion of preformed BK and/or possibly mechanoregulated Ca²⁺ channels into the apical membrane, we examined the effect of slowing this process by performing transport studies at room temperature (22°C) instead of 37°C (64, 74), conditions that did not affect flow stimulation of net Na absorption in CCDs (35). In contrast to the approximately threefold increase in net K secretion detected in control CCDs subject to a fivefold increase in luminal flow rate at 37°C, a reduction in ambient temperature to 22°C inhibited flow-stimulated net K secretion (−1.8 ± 1.3 to −1.3 ± 2.0 pmol·min⁻¹·mm⁻¹; n = 5; P = not significant; Fig. 6) as the luminal flow rate was increased from 1.1 ± 0.1 to 5.5 ± 0.3 nl·min⁻¹·mm⁻¹. However, CCDs perfused at room temperature (n = 6) also failed to exhibit a typical flow-induced [Ca²⁺]i transient (Fig. 5C).

Finally, to test whether an increase in luminal flow rate stimulates exocytic trafficking of BK and/or Ca²⁺ channels from the TGN to the plasma membrane, we examined the effect of BFA (5 μg/ml) on flow-stimulated net K secretion in the CCD. Whereas flow stimulation of net Na absorption was not significantly affected by pretreatment with this agent (35), BFA significantly inhibited flow-stimulated net K secretion (−12.3 ± 4.4 to −15.0 ± 7.4 pmol·min⁻¹·mm⁻¹; n = 4; P = not significant) as the tubular flow rate was increased from 1.0 ± 0.1 to 5.0 ± 0.3 nl·min⁻¹·mm⁻¹ (Fig. 4). Pretreatment of CCDs (n = 4) with BFA did not alter their ability to respond to an acute increase in luminal flow rate with a rapid increase in [Ca²⁺]i (Fig. 5D). These data are consistent with a requirement for exocytic trafficking of channels or associated proteins from the TGN to the plasma membrane in response to an increase in luminal flow rate.

Flow-stimulated K secretion requires Na absorption. To determine the contribution of electrogenic Na absorption to flow-stimulated net K secretion, we measured the effects of an increase in luminal flow rate from 1.0 ± 0.1 to 4.4 ± 0.3 nl·min⁻¹·mm⁻¹ on net K secretion in microperfused rabbit CCDs pretreated with 5 μM benzamil, an agent that inhibits baseline and flow-stimulated net Na absorption (35). Our
results (Fig. 7) demonstrate that flow-stimulated net K secretion is absent (0.1 ± 0.044 pmol·min⁻¹·mm⁻¹; n = 5; P = not significant) in CCDs pretreated with benzamil, underscoring the critical importance of Na absorption in flow-stimulated net K secretion.

DISCUSSION

An acute increase in tubular fluid flow rate in the microperfused rabbit CCD leads to a biphasic response: a prompt transient increase in 

\[ [\text{Ca}^{2+}]_i \]

from 100 to 350 nM within 10 s, followed by a gradual decay to a plateau 

\[ [\text{Ca}^{2+}]_i \]

value that significantly exceeds baseline for at least 10 min during a period of sustained high flow (Fig. 2A), as we previously reported (31). This observation, in light of our previous finding that flow-stimulated net K secretion in the CCD is sensitive to IBX (72), a specific inhibitor of BK but not SK channels, led us to conclude that flow activates Ca²⁺- and/or stretch-activated BK channels.

In general, stimulus-induced increases in 

\[ [\text{Ca}^{2+}]_i \]

are mediated by Ca²⁺ influx through plasma membrane Ca²⁺ channels (e.g., mechano-, voltage-, and/or ligand-gated) and exchangers, release from intracellular stores through ryanodine- and IP₃-

Fig. 6. Effect of reduction in perfusion temperature from 37°C to room temperature (22°C) on flow-stimulated net K secretion in microperfused rabbit CCDs. Net K secretion was measured at tubular flow rates of 1 and 5 nl·min⁻¹·mm⁻¹ at 37°C (n = 7) or room temperature (RT; n = 5). Means ± SE. *P < 0.05 vs. transport rate at 1 nl·min⁻¹·mm⁻¹ in the same CCDs. #P < 0.05 vs. transport rate at the same flow rate in CCDs perfused at 37°C.

Fig. 7. Flow-stimulated net K secretion is dependent on net Na absorption in microperfused rabbit CCDs. Net K secretion was measured at tubular flow rates of 1 and 5 nl·min⁻¹·mm⁻¹ in the absence (control, C; n = 7) or presence of 5 μM benzamil (BZ; n = 5), a selective inhibitor of ENaC. Means ± SE. *P < 0.05 vs. transport rate at 1 nl·min⁻¹·mm⁻¹ in the same CCDs. #P < 0.05 vs. transport rate at the same flow rate in control CCDs.
sensitive channels, and signaling through specific Ca\(^{2+}\)-transducer proteins (5). The flow-induced increase in [Ca\(^{2+}\)]\(_i\) in the microperfused rabbit CCD appears to be, at least in part, to both mobilization of internal stores and external Ca\(^{2+}\) influx (31). Although the peak and sustained elevations in global [Ca\(^{2+}\)], elicited by flow in the CCD are within range of those considered necessary to activate BK channels in the CCD (>100 nM) (40) and CNT (500 nM) (54), the local concentration of Ca\(^{2+}\) in the immediate vicinity of the channels already resident at the apical membrane is likely to be much greater, but has not yet been measured. The results of the present study underscore the importance of this flow-induced increase in [Ca\(^{2+}\)]\(_i\) in flow stimulation of net K secretion. Prevention of the flow-induced [Ca\(^{2+}\)]\(_i\) transient by pretreatment of cells with BAPTA-AM to chelate intracellular Ca\(^{2+}\) store release (31) abolished flow-stimulated K secretion (Figs. 1 and 3).

Critical to flow-stimulated net K secretion is luminal Ca\(^{2+}\) entry. We previously reported that elimination of Ca\(^{2+}\) from the luminal perfusate completely inhibited the plateau elevation of [Ca\(^{2+}\)]\(_i\), that followed the flow-induced rapid [Ca\(^{2+}\)]\(_i\) transient (31), suggesting that this luminal Ca\(^{2+}\)-dependent plateau represents store-operated Ca\(^{2+}\) entry. We now show that flow-stimulated net K secretion is also dependent on apical Ca\(^{2+}\) entry; removal of Ca\(^{2+}\) from the luminal perfusate and thus prevention of luminal Ca\(^{2+}\) entry abrogated flow stimulation of net K secretion. The identity of the apical Ca\(^{2+}\) entry pathway remains to be defined. One likely candidate is the transient receptor potential vanilloid 4 (Trpv4) channel, which is highly expressed in the distal nephron and collecting duct (14, 57), although its precise cellular localization remains controversial. While immunodetectable Trpv4 has been shown to colocalize with PACSIN 3, a protein proposed to block dynamin-mediated endocytosis, in the luminal membrane of tubule cells (13), studies using a different antibody revealed predominant immunoreactivity in the basolateral membrane of intercalated cells (57). The Trpv4 channel functions not only as an osmosensor (30, 53) but also responds to mechanical stress (17, 30) and heat (22, 68). CCDs isolated from Trpv4 knockout mice and microperfused in vitro fail to exhibit flow dependence of net K secretion and Na absorption, in contrast to their wild-type counterparts (55). Of particular relevance to the present study are the findings that Trpv4-overexpressing HEK293 cells exhibit flow/shear sensitivity at 37°C but not at room temperature, and activators of Trpv4 at 37°C have minimal or no effect on channel activation at a room temperature of 22–24°C (17). The observation that temperature is a critical regulator of Trpv4 channel gating may explain our finding that flow did not induce an increase in [Ca\(^{2+}\)]\(_i\) in CCDs perfused at room temperature (Fig. 5C). Another candidate Ca\(^{2+}\) channel deserving of investigation is TRPC6, a nonselective cation channel that directly senses membrane stretch induced by mechanical or osmotic stimuli (51). Immunodetectable TRPC6 is found in principal but not intercalated cells of the rodent distal nephron (18). Finally, polycystin 1 (PC1, TRPP1) and polycystin 2 (PC2, TRPP2), the gene products of PKD1 and PKD2 that are mutated in autosomal dominant PKD (ADPKD), have been proposed to form a mechanosensitive ion channel. PC1 interacts with PC2, a Ca\(^{2+}\)-permeable channel (23, 59). Cumulative evidence suggests that conformational changes of PC1 in the apical cilium of renal epithelial cells transduce a mechanical signal into a chemical response by activating associated PC2 channels; local Ca\(^{2+}\) influx into the cilium subsequently triggers internal Ca\(^{2+}\) release (37).

The Ca\(^{2+}\) dependence of sustained flow stimulation of net K secretion is at odds with the observation that functional BK channels studied by patch-clamp analysis can be activated by stretch in rabbit CCD intercalated cells even after chelation of free Ca\(^{2+}\) with EGTA in the pipette or the bath solutions (40). The latter observation implies that stretch activation of these channels is not mediated by enhanced Ca\(^{2+}\) entry into the cell or internal release, but that the channel is in itself mechanosensitive, and is directly responsive to membrane deformation. If so, flow activation of the luminal BK channel may be the initial event, leading to membrane hyperpolarization, in turn facilitating Ca\(^{2+}\) influx through Ca\(^{2+}\)-permeable channels, as has been demonstrated in endothelial cells (39). Our observation that IBX inhibits flow-stimulated net K secretion (71) but not the flow-induced [Ca\(^{2+}\)]\(_i\) transient (Fig. 2C) argues against the latter hypothesis. However, it should be noted that IBX-treated CCDs, like those treated with lumicolchicine and BFA, failed to exhibit a typical plateau elevation of [Ca\(^{2+}\)]\(_i\) (Fig. 2). To the extent that lumicolchicine had no effect on flow stimulation of net K secretion, the significance of the loss of sustained luminal Ca\(^{2+}\) entry on BK channel activation remains to be determined.

Alternative splice variants of the COOH terminus of the α-subunit and expression of distinct isoforms of the β-subunit result in channels that differ in their activation by [Ca\(^{2+}\)]\(_i\), membrane potential and stretch, inhibitor sensitivity, and as suggested in more recent studies, subcellular localization and association with interacting proteins (10, 33, 58, 66). BK channel β1-4 subunits have been identified at the mRNA level in mammalian kidney (36, 61, 70) and appear to be differentially regulated along the nephron. In heterologous systems, coexpression of β1 with α increases the Ca\(^{2+}\) sensitivity and charybdoxin binding affinity of the channel (15, 33). Although β1 would be a logical subunit to comprise the CCD BK channel, its localization is restricted to the CNT (43) where it plays a role in flow-stimulated urinary K secretion (42). Coexpression of β2 (61) or β3 (6, 61, 73) with the α-subunit results in complete or partial, respectively, inactivation of channel currents. β4 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 IBX association rates (6, 34, 65). The inhibitory nature of the β2 and β3 subunits suggests they are unlikely candidate subunits for the BK conductance in the native CCD. The composition of the native channel in the CCD, whether comprised of an α-subunit alone, an α-subunit associated with a nonglycosylated (IBX-sensitive) β4 subunit, or an as yet undescribed β-subunit, remains to be clarified.

A flow-induced increase in BK-mediated net K secretion may be due to an increase in number of channels at the apical membrane or an increase in open probability of channels already resident at that membrane. The observation of significant intracellular localization of immunodetectable BK channel α-subunit in the CCD (36) raised the possibility that alterations in channel activity at the cell surface may occur by exocytic insertion of intracellular reserves of channels into the apical membrane. Cumulative evidence suggests that increases
in [Ca^{2+}], participate in vesicular trafficking by triggering the microtubule-dependent exocytic movement of vesicles to their target membranes (1, 9). In the distal nephron, an acute elevation of Pco_2 rapidly stimulates the exocytic fusion of vesicles containing H^{+}-ATPases with the luminal membrane of intercalated cells (50), a response induced by a transient increase in [Ca^{2+}]. (12). Similarly, the adaptation of the collecting duct to in vivo and in vitro chronic metabolic acidosis is manifest by an increase in apical H^{+} pumps and H^{+} secretion (47, 60). Studies in the outer medullary collecting duct (OMCD) reveal that this adaptation depends on exocytosis, cytoskeletal integrity, and activation of calmodulin/CaMK-associated intracellular Ca^{2+} signaling (60). Finally, in rat inner medullary collecting duct, vasopressin binding to the V_2 receptor triggers intracellular Ca^{2+} mobilization, which is required for translocation and fusion of intracellular vesicles containing aquaporin-2 (AQP2) into the apical plasma membrane and an increase in osmotic water permeability; pretreatment of microperfused tubules with BAPTA-AM clamped [Ca^{2+}], (i.e., inhibited the AVP-induced Ca^{2+} mobilization) and prevented apical exocytosis (75). Also necessary for AQP2 apical targeting are interactions of AQP2-containing vesicles with actin and the microtubule cytoskeleton (7, 62).

Three lines of evidence suggest that flow-stimulated BK channel-mediated net K secretion requires, in part, an intact microtubule network and/or exocytic insertion of preexisting channels into the apical membrane. First, we previously reported that immunodetectable BK channel α-subunit is localized predominantly to a subapical region in CCD cells but is more highly expressed at the apical membrane in tubules isolated from high K-fed animals (36), consistent with diet/hormone-mediated translocation of cytoplasmic channels to the apical membrane. Second is our observation that colchicine, an inhibitor of tubulin polymerization (35), and reduction of ambient temperature, which slows exocytosis in epithelial cells (64, 74), totally blocked the flow-stimulated increase in net K secretion (Figs. 4 and 6). However, the finding that colchicine and a reduction in ambient temperature also blocked the flow-induced increase in [Ca^{2+}], (Fig. 5) suggests that 1) the inhibition of flow-stimulated K secretion by these agents may simply reflect the loss of the flow-induced increase in [Ca^{2+}], and/or 2) an intact microtubule network and exocytosis are necessary for Ca^{2+} entry channels to gate open and/or gain access to the apical membrane, respectively. In support of the latter notion is the recent report that HEK-293 cells expressing GFP-TRPM7, studied by TIRF imaging at 22°C, respond to shears in the range of 5–15 dynes/cm^2 with the rapid (within 2 min) accumulation of functional channels at the plasma membrane, consistent with accelerated fusion of TRPM7-containing vesicles with the plasma membrane (38). Third, the blunted effect of an increase in tubular flow rate on net K secretion in BFA-treated CCDs is consistent with mechano-induced channel trafficking between the TGN and the apical plasma membrane along the late secretory pathway.

The expression of immunodetectable apical BK α-subunits (36, 72) and conducting BK channels (40) in intercalated cells exceeds that observed in principal cells. However, the mechanism by which intercalated cells, characterized by low levels of Na-K-ATPase activity (4), might sustain high rates of flow-stimulated K secretion is uncertain. Our observation that benzamil completely abolished baseline and flow-stimulated net K secretion (Fig. 7) confirms the dependence of K secretion on Na absorption. The recent report that BK channel activity in principal cells is significantly increased by inhibition of both ERK and P38 (29) suggests that these cells may mediate stretch- and/or Ca^{2+}-activated net K secretion under certain conditions. Alternatively, flow-stimulated K secretion could be mediated by intercalated cells, driven by the high intracellular K concentration (49) and lumen negative potential; the latter enhanced by the flow-stimulated increase in ENaC activity (48) in adjacent principal cells, transmitted to intercalated cells by "intraepithelial current flow" (27). In support of the concept that intercalated and principal cells can interact via "intraepithelial current flow" is the observation that inhibition of electrogenic Na transport by amiloride in principal cells hyperpolarizes voltage across the apical membrane of adjacent intercalated cells in microperfused rabbit CCDs (27). At the tubular level, changes in Na transport lead to alterations in urine acidification (28).

In sum, our data suggest that flow-stimulated BK channel-mediated net K secretion involves activation of a signal cascade that requires an increase in [Ca^{2+}], microtubule integrity, and exocytic insertion of preformed channels into the apical membrane. The molecular mechanisms whereby this cascade allows for a sustained increase in net K secretion under conditions of high urinary flow remain to be determined.

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REFERENCES

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