Cyclic AMP-induced \( K^+ \) secretion occurs independently of \( Cl^- \) secretion in rat distal colon

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Sandle GI, Rajendran VM. Cyclic AMP-induced \( K^+ \) secretion occurs independently of \( Cl^- \) secretion in rat distal colon. Am J Physiol Cell Physiol 303: C328–C333, 2012. First published May 30, 2012; doi:10.1152/ajpcell.00099.2012.—cAMP induces both active \( Cl^- \) and active \( K^+ \) secretion in mammalian colon. It is generally assumed that a mechanism for \( K^+ \) exit is essential to maintain cells in the hyperpolarized state, thus favoring a sustained \( Cl^- \) secretion. Both Kcnn4c and Kcnma1 channels are located in colon, and this study addressed the questions of whether Kcnn4c and/or Kcnma1 channels mediate cAMP-induced \( K^+ \) secretion and whether cAMP-induced \( K^+ \) secretion provides the driving force for \( Cl^- \) secretion. Forskolin (FSK)-enhanced short-circuit current (indicator of net electrogenic ion transport) and \( K^+ \) fluxes were measured simultaneously in colonic mucosa under voltage-clamp conditions. Mucosal Na \( ^+ \)/H\( ^+ \)secretion provides the driving force for \( Cl^- \) secretion in rat distal colon. In the presence of mucosal Na \( ^+ \)orthovandate (P-type ATPase inhibitor) inhibited active \( K^+ \) absorption normally present in rat distal colon. In the presence of mucosal Na \( ^+ \)orthovandate, serosal FSK induced both \( K^+ \) and \( Cl^- \) secretion. FSK-induced \( K^+ \) secretion was not inhibited by either mucosal or serosal 1-[(2-chlorophenyl) diphenylmethyl]-1H-pyrazole (TRAM-34; a Kcnn4 channel blocker), 2) inhibited (92%) by mucosal iberiotoxin (Kcnma1 channel blocker), and 3) not affected by mucosal cystic fibrosis transmembrane conductance regulator inhibitor (CFTRinh-172). By contrast, FSK-induced \( Cl^- \) secretion was completely inhibited by serosal TRAM-34, 2) not inhibited by either mucosal or serosal iberiotoxin, and 3) not affected by mucosal CFTRinh-172. These results indicate that cAMP-induced colonic \( K^+ \) secretion is mediated via Kcnma1 channels located in the apical membrane and most likely contributes to stool \( K^+ \) losses in secretory diarrhea. On the other hand, cAMP-induced colonic \( Cl^- \) secretion requires the activity of Kcnn4b channels located in the basolateral membrane and is not dependent on the concurrent activation of apical Kcnma1 channels.

Fluid secretion; short-circuit current; potassium fluxes; adenosine 3’,5’-cyclic monophosphate

**ACTIVE CHLORINE SECRETION** is the primary driving force for fluid secretion in infectious diarrheas such as cholera (4, 5). Active \( Cl^- \) secretion requires both basolateral \( Cl^- \) uptake and apical \( Cl^- \) exit mechanisms and a \( K^+ \) extrusion pathway that maintains cells in a hyperpolarized state as well as delivering \( K^+ \) ions to the Na–K-CI cotransporter (NKCC) and Na-K-ATPase located in the basolateral membrane. NKCC mediates \( Cl^- \) uptake across the basolateral membrane, whereas cystic fibrosis transmembrane regulator (CFTR) mediates active \( Cl^- \) secretion across the apical membrane. Intermediate-conductance, \( Ca^{2+} \)-activated \( K^+ \) channels localized to the basolateral membrane have been shown to provide the driving force for agonist (\( Ca^{2+} \), cAMP)-induced \( Cl^- \) secretion in human and mouse colon (22, 23). Molecular studies have identified intermediate conductance \( K^+ \) channels in different species, which are variously referred to as intermediate conductance \( K^+ \) channel, mouse intermediate conductance \( K^+ \) channel, small conductance \( K^+ \) channel 4, \( Ca^{2+} \)-activated \( K^+ \) channel 3.1, and Kcnn4 (14, 17, 30). Immunofluorescence studies have localized Kcnn4-like proteins to both the apical and basolateral membranes of human, guinea pig, and rat colon (3, 10, 11). The splice variants Kcnn4c and Kcnn4b have been shown to encode apical and basolateral Kcnn4 channels in rat distal colon (3). Activation of Kcnn4c with 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazole-2-one (DC-EBIO; a Kcnn4 channel opener and CFTR activator) has been shown, in part, to provide the driving force for \( Cl^- \) secretion in rat distal colon (16).

Electrophysiological and immunofluorescence studies have identified Kcnma1 channels in the apical membranes of mammalian (including human) colon (3, 15). cAMP has been shown to activate apical Kcnma1 channels in rat colon, as well as stimulating \( K^+ \) secretion via Kcnma1 channels in mouse colon (21, 26). The aims of the present study were to determine whether Kcnma1 and/or Kcnma4 channels mediate cAMP-induced \( K^+ \) secretion and whether cAMP-induced \( K^+ \) secretion provides the driving force for \( Cl^- \) secretion in rat distal colon. The results indicate that apical Kcnma1 channels (but not Kcnma4 channels) mediate cAMP-induced \( K^+ \) secretion, whereas basolateral Kcnn4b channels (but not apical Kcnma1 or Kcnma4 channels) provide the driving force for cAMP-induced \( Cl^- \) secretion. We conclude that cAMP-induced \( K^+ \) and \( Cl^- \) secretion occurs independently and that the cAMP-induced \( K^+ \) secretion via Kcnma1 channels may contribute to high fecal \( K^+ \) losses in some cases of secretory diarrhea.

**METHODS**

*Animals.* Nonfasting normal male Sprague-Dawley rats (201–225 g) were maintained on standard rat chow. Animals were given food and water ad libitum. The experimental protocols used in these studies were approved by the West Virginia University Institutional Animal Care and Use Committee.

*Ion flux studies.* \( ^{85}Rb \) (\( K^+ \) surrogate; PerkinElmer, Billerica, MA) fluxes, short-circuit current (\( I_sc \); a measure of net electrogenic ion transport), and trans-epithelial conductance (\( G \)) were measured across colonic mucosa bathed in symmetrical Ringer solution (see below) and mounted under voltage-clamp conditions at 0 mV in EasyMount Ussing chambers (Physiological Instruments, San Diego, CA), as previously described (16). Thus, by removing the transepithelial electrical potential difference that would be present under open-circuit (mimicking in vivo) conditions, the net flux of the ionic species under study reflected its active (i.e., potential independent) transport. In brief, distal colons excised from euthanized rats were flushed with ice-cold saline. The distal colon was opened along the mesenteric border, and mucosal sheets were gently separated from serosal muscular layers. Two distal (1 cm proximal to rectum) segments obtained by 10.220.33.6 on April 13, 2017 http://ajpcell.physiology.org/ Downloaded from http://www.ajpcell.org
from each animal were mounted on to snap wells with an opening of 1.12 cm². The snap wells placed in the sliders were inserted into the chambers, and both sides were bathed with equal volumes (5 ml) of Ringer solution containing (in mM) 115 NaCl, 25 NaHCO₃, 2.4 K₂HPO₄, 0.4 KH₂PO₄, 1.2 CaCl₂, 1.2 MgCl₂, and 10 glucose, titrated to pH 7.4. Bathing solutions were maintained at 37°C and gassed with 5% CO₂-95% O₂. The Iₑ and G were recorded every 20 s using an automated multichannel voltage/current clamp instrument (Physiological Instruments). Positive Iₑ represented active Cl⁻ secretion, whereas negative Iₑ represented active K⁺ secretion.

For K⁺ flux studies, a trace of ⁸⁶RbCl (1 μCi/chamber) was added to either the mucosal (m) or serosal (s) bath solutions. After a 45-min equilibration period, mucosal-to-serosal (m-s) and serosal-to-mucosal (s-m) ⁸⁶Rb⁺ fluxes were measured under voltage-clamp conditions in different tissues. Net fluxes were calculated from the difference between m-s and s-m fluxes in tissue pairs that were matched based on differences in basal conductance of <10%. Positive and negative values represent active absorption and active secretion, respectively.

The K⁺ fluxes were measured over 15-min periods. At the end of each period, 0.5-ml samples were withdrawn from the bathing solution opposite to the “hot” side (i.e., isotope-containing side). Following sample removal, 0.5 ml regular Ringer was added to maintain the bath volume. Basal fluxes were measured immediately following an equilibration period. Following the basal flux period, forskolin (FSK; 10 μM), an adenylate cyclase inhibitor that increases intracellular cAMP levels, was added to the serosal bath. In addition, 1 mM Na⁺ orthovanadate (VO₄; a P-type ATPase inhibitor) to the mucosal bath solution to inhibit apical H⁺,K⁺-ATPase. In the same way that FSK stimulated Cl⁻ secretion in the absence of VO₄ (Fig. 1A), the simultaneous addition of FSK to the serosal bath solution and VO₄ to the mucosal bath solution (FSK/VO₄) also enhanced Cl⁻ secretion (Fig. 2A). However, in contrast to the persistence of net K⁺ absorption in the absence of VO₄ (Fig. 1B), adding FSK/VO₄ combination elicited net K⁺ secretion (basal vs. FSK/VO₄: 0.6 ± 0.1 vs. −0.7 ± 0.1 μeq/h·cm²; P < 0.001) (Fig. 2B). Active K⁺ secretion induced by FSK/VO₄ reflected both a decrease in the m-s K⁺ flux (basal vs. FSK/VO₄: 1.1 ± 0.2 vs. 0.3 ± 0.1; P < 0.001) and an increase in the s-m K⁺ flux (basal vs. FSK/VO₄: 0.5 ± 0.04 vs. 0.9 ± 0.1; P < 0.001) (Fig. 2B).

Statistics. Values presented are means ± SE of six tissue pairs obtained from different rat distal colons. Statistical analyses were performed using unpaired or paired Student’s t-test or Bonferroni’s one-way ANOVA post-hoc test using Originpro 8.0 (OriginLab, Northampton, MA). P < 0.05 was considered to be statistically significant.

RESULTS

In initial studies, the effect of serosal addition of 10 μM FSK (an adenylate cyclase inhibitor that increases intracellular cAMP levels) on Iₑ and K⁺ fluxes was examined in rat distal colon (Fig. 1). FSK stimulated Iₑ, indicating an increase in Cl⁻ secretion (Fig. 1A). As shown previously, net K⁺ absorption was present under basal conditions (Fig. 1B). FSK significantly decreased net K⁺ absorption from 1.5 ± 0.2 to 0.7 ± 0.2 μeq/h·cm² (P < 0.001) (Fig. 1B) by increasing the s-m K⁺ flux from 0.3 ± 0.04 to 0.8 ± 0.02 μeq/h·cm² (P < 0.001) without changing the m-s K⁺ flux (Fig. 1B).

Although FSK enhanced the s-m K⁺ flux, it did not elicit active K⁺ secretion in the distal colon (Fig. 1B). It is possible that H⁺,K⁺-ATPase located in the apical membrane mediated a K⁺ absorptive (m-s) flux that masked FSK-induced K⁺ secretion (8, 19). Experiments were therefore performed to evaluate K⁺ fluxes in the absence of active K⁺ absorption. These involved adding 1 mM Na⁺ orthovanadate (VO₄; a P-type ATPase inhibitor) to the mucosal bath solution to inhibit apical H⁺,K⁺-ATPase. In the same way that FSK stimulated Cl⁻ secretion in the absence of VO₄ (Fig. 1A), the simultaneous addition of FSK to the serosal bath solution and VO₄ to the mucosal bath solution (FSK/VO₄) also enhanced Cl⁻ secretion (Fig. 2A). However, in contrast to the persistence of net K⁺ absorption in the absence of VO₄ (Fig. 1B), adding FSK/VO₄ combination elicited net K⁺ secretion (basal vs. FSK/VO₄: 0.6 ± 0.1 vs. −0.7 ± 0.1 μeq/h·cm²; P < 0.001) (Fig. 2B). Active K⁺ secretion induced by FSK/VO₄ reflected both a decrease in the m-s K⁺ flux (basal vs. FSK/VO₄: 1.1 ± 0.2 vs. 0.3 ± 0.1; P < 0.001) and an increase in the s-m K⁺ flux (basal vs. FSK/VO₄: 0.5 ± 0.04 vs. 0.9 ± 0.1; P < 0.001) (Fig. 2B).

These results indicate that FSK stimulates both active Cl⁻ and K⁺ secretion.

Fig. 1. Effect of forskolin (FSK) on short-circuit current (Iₑ) and K⁺ fluxes in rat distal colon. A: time course of Iₑ in the absence and in the presence of FSK. FSK (10 μM) was added to serosal bath (arrow). B: mucosal to serosal (m-s), serosal to mucosal (s-m), and net K⁺ fluxes (net) were measured in the absence (white bars) and in the presence (black bars) of FSK. Both Iₑ and K⁺ fluxes were measured under voltage-clamp conditions. *P < 0.001, compared with respective fluxes in the absence of FSK/VO₄.

Fig. 2. Effect of FSK in the presence of mucosal Na⁺ orthovanadate (VO₄) on Iₑ and K⁺ fluxes in rat distal colon. A: time course of Iₑ in the absence and in the presence of FSK and VO₄. VO₄ (1 mM) was added to mucosal bath, whereas 10 μM FSK was added to serosal bath (FSK/VO₄). B: m-s, s-m, and net K⁺ fluxes were measured in the absence (white bars) and in the presence (black bars) of FSK. Both Iₑ and K⁺ fluxes were measured under voltage-clamp conditions. *P < 0.001, compared with respective fluxes in the absence of FSK/VO₄.
Fig. 3. Effect of 1,2-[2-chlorophenyl]-diphenylmethy]-1H-pyrazole (TRAM-34) on FSK-induced $I_{sc}$ and active $K^+$ secretion in rat distal colon. A: time course of $I_{sc}$ in the absence and in the presence of FSK and VO$_4$. VO$_4$ (1 mM) was added to mucosal bath, whereas 10 $\mu$M FSK was added to serosal bath (FSK/VO$_4$). $I_{sc}$ in the presence of FSK/VO$_4$ was also measured in presence of 50 $\mu$M mucosal [TRAM-34 (m)] and 500 $\mu$M serosal [TRAM-34 (s)]. Arrows indicate the time of addition of respective drug. B: m-s, s-m, and net $K^+$ fluxes were measured in the presence of FSK/VO$_4$ (white bars). The $K^+$ fluxes were also measured in the presence of mucosal [TRAM-34 (m); gray bars] and serosal [TRAM-34 (m/s); black bars] TRAM-34. Both $I_{sc}$ and $K^+$ fluxes were measured under voltage-clamp conditions.

secretion and active $K^+$ secretion in rat distal colon. All further studies to characterize the FSK-induced active $K^+$ secretory process were therefore performed in the presence of VO$_4$.

Additional studies were performed to identify the $K^+$ channels involved in FSK-induced $K^+$ secretion and to determine whether FSK-induced $K^+$ secretion provides the driving force for FSK-induced Cl$^-$ secretion. To this end, we used TRAM-34 (a Kcnn4 channel blocker) and IbTX (a Kcnma1 channel blocker). The mucosal addition of 50 $\mu$M TRAM-34 did not significantly inhibit either FSK-induced Cl$^-$ secretion (Fig. 3A) or FSK-induced $K^+$ secretion (Fig. 3B). However, in the presence of mucosal TRAM-34, a serosal addition of 500 nM TRAM-34 completely inhibited FSK-induced Cl$^-$ secretion (Fig. 3A) but had no effect on FSK-induced $K^+$ secretion (Fig. 3B). In contrast to TRAM-34, neither the mucosal nor the serosal addition of 100 nM IbTX inhibited FSK-induced Cl$^-$ secretion (Fig. 4A), whereas mucosal IbTX completely inhibited FSK-induced $K^+$ secretion (Fig. 4B). These observations indicate that 1) $K^+$ efflux via basolateral Kcnn4 (Kcnn4b) channels, but not via apical Kcnn1 channels, provide the driving force for FSK-induced Cl$^-$ secretion; 2) the apical Kcnn1 channels, but not Kcnn4c channels, mediate FSK-induced $K^+$ secretion; and 3) FSK may activate basolateral Kcnn4b, but not apical Kcnn4c $K^+$ channels in rat distal colon.

Since Chrom-293B-sensitive cAMP-activated $K^+$ channels (KCNQ1) have been identified in the basolateral membranes of colonic crypts (31), we examined the effect of Chrom-293B on FSK-induced Cl$^-$ and $K^+$ secretion (Fig. 5). Mucosal Chrom-293B progressively inhibited FSK-induced Cl$^-$ secretion to 52%. In the continued presence of mucosal Chrom-293B, serosal Chrom-293B had no further effect on FSK-induced Cl$^-$ secretion (Fig. 5A). Serosal Chrom-293B alone (i.e., in the absence of mucosal Chrom-293B) inhibited FSK-induced Cl$^-$ secretion by $\sim$40% (data not shown). The fact that mucosal plus serosal Chrom-293B or serosal Chrom-293B alone inhibited Cl$^-$ secretion to similar extents suggests that Chrom-293B freely permeated both the mucosal and the serosal membranes. In contrast to Cl$^-$ secretion, FSK-induced $K^+$ secretion was not significantly altered by Chrom-293B (Fig. 5B). The observations that complete and partial inhibition of FSK-enhanced Cl$^-$ secretion by TRAM-34 (Fig. 3A) and Chrom-293B (Fig. 5A), respectively, suggest that it is likely that either the Chrom-293B-sensitive KCNQ1 expressed in basolateral membrane is also sensitive to TRAM-34 or that more than one Kcnn4 isoform may be expressed in basolateral membranes, one of which may be sensitive to Chrom-293B.

Our next step was to explore the possibility that Cl$^-$ and $K^+$ secretion might be interdependent by examining the effects of a Cl$^-$ channel blocker on $K^+$ secretion and a $K^+$ channel opener on Cl$^-$ secretion. Mucosal addition of 100 $\mu$M CFTRinh-172 (a CFTR channel blocker) completely inhibited the FSK-enhanced Cl$^-$ secretion (Fig. 6A) but had no effect on FSK-induced $K^+$ secretion (Fig. 6B). In additional experiments, in which 1 mM VO$_4$ was present in the mucosal bath solution, which had no effect on $I_{sc}$ (Fig. 7A), the mucosal addition of 10 $\mu$M BMS (a Kcnma1 channel opener) decreased $I_{sc}$, consistent with the activation of electrogenic $K^+$ secretion (Fig. 7A). Mucosal VO$_4$ having completely inhibited net $K^+$.

Fig. 4. Effect of iberiotoxin (IbTX) on FSK-induced $I_{sc}$ and active $K^+$ secretion in rat distal colon. A: time course of $I_{sc}$ in the absence and in the presence of FSK and VO$_4$. VO$_4$ (1 mM) was added to mucosal bath, whereas 10 $\mu$M FSK was added to serosal bath (FSK/VO$_4$). $I_{sc}$ in the presence of FSK/VO$_4$ was also measured in additional presence of 100 nM mucosal [IbTX (m)] and serosal [IbTX (m/s)] IbTX. B: m-s, s-m, and net $K^+$ fluxes were measured in the presence of FSK/VO$_4$ (white bars). The $K^+$ fluxes were also measured in the presence of mucosal [IbTX (m); gray bars] and serosal [IbTX (m/s); black bars] IbTX. Both $I_{sc}$ and $K^+$ fluxes were measured under voltage-clamp conditions. *$P < 0.001$, compared with respective fluxes in the absence of IbTX.
absorption (Fig. 7B), the subsequent mucosal addition of BMS-induced net K⁺ secretion. These results provide strong evidence that FSK-induced Cl⁻ secretion mediated via apical CFTR proceeds independently of K⁺ secretion mediated via apical Kcnma1 channels. As a corollary, K⁺ secretion via apical Kcnma1 channels do not provide the driving force for FSK-induced Cl⁻ secretion in rat distal colon.

**DISCUSSION**

In the present study we have shown that cAMP-induced K⁺ secretion via apical Kcnma1 channels and cAMP-induced Cl⁻ secretion via apical CFTR are regulated independently (Fig. 8). Furthermore, basolateral Kcnm4 (Kcnm4b) channels, but not apical Kcnm4 (Kcnm4c) or Kcnma1 channels, provide the driving force for the cAMP-induced Cl⁻ secretion in rat distal colon.

In infectious diarrheas such as cholera, movement of water into the gut lumen occurs secondary to active Cl⁻ secretion. Active Cl⁻ secretion requires the coordinated regulation of apical Cl⁻ channels and the NKCC, Na,K-ATPase, and K⁺ channels located in the basolateral membrane. Chloride ions entering cells via basolateral NKCC exits via apical CFTR. Sustained Cl⁻ secretion also requires the activity of K⁺ channels, which keep cells hyperpolarized, thus providing the driving force for apical Cl⁻ exit (4, 5). Previous studies have implicated a role for basolateral Kcnm4 channels in providing the driving force for active Cl⁻ secretion (22, 23), but apical Kcnm4c and Kcnma1 channels have been identified in mouse, rat and human colon (3, 10, 11, 16, 21, 24–26), and apical Kcnm4c channel activity appears to be involved in electrogenic Cl⁻ secretion (16). However, it has been unclear whether active K⁺ secretion mediated via apical Kcnma1 channels also contributes to the driving force required for Cl⁻ secretion, as well as to the excessive stool K⁺ losses seen in certain types of secretory diarrhea.

The observation that the mucosal addition of BMS (a Kcnma1 channel opener) induced active K⁺ secretion points to the presence of apical Kcnma1 channels in rat distal colon (Fig. 7B), as shown by previous patch-clamp studies in this epithelium (6, 24). Although Kcnma1 channels are localized to the apical membrane, active K⁺ secretion is not present under basal condition in rat distal colon (Fig. 7B). This situation differs from that in mouse distal colon in which apical Kcnma1 channels are present along the crypt axis and mediate both basal and Ca²⁺-activated K⁺ secretion (26). Based on the different patterns of apical Kcnma1 channel distribution along the surface-crypt axis, distinct patterns of cAMP-activated colonic K⁺ secretion have been identified in crypt cells in different mouse strains (9) and in surface cells in rat colon (21). Therefore, it is likely that the absence or presence of basal K⁺ secretion may reflect different expression patterns of Kcnma1 channels in rat colon and mouse colon, respectively.

Although both Kcnm4c and Kcnma1 channels are present at the apical membrane of rat distal colon, cAMP-induced active K⁺ secretion only via Kcnma1 channels (Fig. 1B). Phosphorylation of apical Kcnma1 channels by the protein kinase A-mediated signaling pathway most likely underlies cAMP-activated colonic K⁺ secretion in rat colon (21), whereas Ba²⁺- and IbTX-sensitive K⁺ secretion in wild-type (Kcnma1⁺/⁺) but not in Kcnma1-deficient (Kcnma1⁻/⁻) animals has been proposed as evidence for the involvement of Kcnma1 channels in cAMP-stimulated K⁺ secretion in mouse distal colon (27). Thus, although K⁺ secretion is absent in rat distal colon under conditions.
basal condition, cAMP-stimulated IbTX-inhibitable K⁺ secretion is entirely mediated by apical Kcnma1 channels.

The present study in rat distal colon demonstrates that although the process of active K⁺ secretion most likely maintains cells in a hyperpolarized state and contributes to the driving force for Cl⁻ secretion, the activation of apical Kcnma1 channels is not in itself required for cAMP-enhanced Cl⁻ secretion. This conclusion is supported by several observations. First, the Kcnma1 channel blocker IbTX inhibited cAMP-stimulated K⁺ secretion, but not cAMP-stimulated Cl⁻ secretion (Fig. 4, A and B). Second, the CFTR blocker CFTRinh-172 inhibited cAMP-stimulated Cl⁻ secretion but not cAMP-stimulated K⁺ secretion (Fig. 6, A and B). Third, serosal TRAM-34 (a Kcnn4 channel blocker) inhibited cAMP-stimulated Cl⁻ secretion, indicating that basolateral Kcnn4 channels provide the driving force for cAMP-stimulated Cl⁻ secretion (Fig. 3A). It is possible that apical Kcnma1 and CFTR channels are located in different cell types, since patch-clamp studies have identified apical Kcnma1 channels in surface cells of rat and human colon (6, 25), whereas apical CFTR is present in crypt cells in these species (1, 18, 28).

The importance of apical Kcnma1 channels in determining stool K⁺ losses has recently been highlighted by several clinical studies. For example, patients with end-stage renal disease exhibit enhanced expression of apical Kcnma1 channels in colonic surface cells (15), which are likely to play an important role in their ability to increase colonic K⁺ losses to maintain K⁺ homeostasis (15). An increase in apical Kcnma1 channel expression also occurs along the entire surface cell-crypt cell axis in active ulcerative colitis (25), which may explain, at least in part, the excessive K⁺ losses and hypokalemia that sometimes occurs in these patients (2, 12–13). Overexpression of apical Kcnma1 channels is likely to underlie massive stool K⁺ losses and profound hypokalemia, which occur in some cases of colonic pseudo-obstruction (29). It also seems likely that hypokalemia complicating the chronic abuse of cAMP-mediated laxatives may reflect excessive stool K⁺ losses via cAMP-stimulated apical Kcnma1 channels (7).

In summary, this study indicates that in rat distal colon, 1) cAMP stimulates Kcnma1 channel-mediated K⁺ secretion and CFTR-mediated Cl⁻ secretion, 2) cAMP-stimulated K⁺ secretion does not provide the driving force for the cAMP-stimulated Cl⁻ secretion, and 3) basolateral Kcnn4b channels provide the driving force for cAMP-stimulated Cl⁻ secretion. Our results also raise the possibility that the K⁺ and Cl⁻ secretory processes regulated by cAMP may be localized in

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**Fig. 8.** Cellular models of cAMP-induced electrogenic Cl⁻ and K⁺ secretion localized in different cell types in rat distal colon. A: coordinated activation of apical CFTR channels and basolateral Na-K-2Cl cotransporter (NKCC) and Kcnn4b channels regulates the cAMP-induced electrogenic Cl⁻ secretion. Kcnn4b channels that mediate K⁺ exit provide the driving force (Drive) for cAMP-induced Cl⁻ secretion, which is inhibited (see text) by blocking either Kcnn4b channels (using TRAM-34) or CFTR (using CFTRinh-172) but not by blocking Kcnma1 channels blocker (using IbTX). B: coordinated activation of apical Kcnma1 channels and basolateral NKCC and chloride channel 2 (CIC-2) (?) regulates cAMP-induced K⁺ secretion, which is inhibited by IbTX but not by CFTRinh-172 or TRAM-34 (see text). CIC2 has been shown to present in basolateral membranes of mammalian colon (20). Direction of transepithelial electrolyte movements (solid arrows), cAMP-activation (dashed arrows), channel blockers (dotted bullets), electrolyte recycling (2-dimensional curvy arrow), and driving force (double-headed arrows) are indicated.

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**Fig. 7.** Effect of BMS-191011 (BMS) on \(I_{sc}\) and K⁺ fluxes in rat distal colon. A: time course of \(I_{sc}\) in the absence and in the presence of 1 mM mucosal VO₄. \(I_{sc}\) in the presence of VO₄ also measured in additional presence of mucosal BMS (10 μM). B: m-s, s-m, and net K⁺ fluxes were measured in the absence (white bars) and in the presence of 1 mM mucosal VO₄ (gray bars). K⁺ fluxes in the presence of VO₄ were also measured in the additional presence of mucosal BMS (black bars). Both \(I_{sc}\) and K⁺ fluxes were measured under voltage-clamp conditions. *P < 0.001, compared with respective fluxes in the absence of VO₄; EP < 0.001, compared with respective fluxes in the presence of VO₄ and absence of BMS.
different cell types. Finally, we propose that cAMP-regulated apical Kcnma1 channels may play a critical role in determining stool K$^+$ losses in some types of secretory diarrhea.

**GRANTS**

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

G.I.S. and V.M.R. conception and design of research; G.I.S. and V.M.R. analyzed data; G.I.S. and V.M.R. interpreted results of experiments; G.I.S. and V.M.R. edited and revised manuscript; G.I.S. and V.M.R. approved final manuscript; V.M.R. performed experiments; V.M.R. prepared figures; V.M.R. drafted manuscript.

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