Secretory modulation of basolateral membrane inwardly rectified K⁺ channel in guinea pig distal colonic crypts

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Li, Yingjun, and Dan R. Halm. Secretory modulation of basolateral membrane inwardly rectified K⁺ channel in guinea pig distal colonic crypts. Am J Physiol Cell Physiol 282: C719–C735, 2002. First published October 24, 2001; 10.1152/ajpcell.00065.2001.—Cell-attached recordings revealed K⁺ channel activity in basolateral membranes of guinea pig distal colonic crypts. Inwardly rectified currents were apparent with a pipette solution containing 140 mM K⁺. Single-channel conductance (γ) was 9 pS at the resting membrane potential. Another inward rectifier with γ of 19 pS was observed occasionally. At a holding potential of −80 mV, γ was 21 and 41 pS, respectively. Identity as K⁺ channels was confirmed after patch excision by changing the bath ion composition. From reversal potentials, relative permeability of Na⁺ over K⁺ (PNa/PK) was 0.02 ± 0.02, with PNa/PK = 1.1 and PCl/PK < 0.03. Spontaneous open probability (Po) of the 9-pS inward rectifier (8Kᵣ) was voltage independent in cell-attached patches. Both a low (Po = 0.09 ± 0.01) and a moderate (Po = 0.41 ± 0.01) activity mode were observed. Excision moved 8Kᵣ to the medium activity mode; Po of 8Kᵣ was independent of bath Ca²⁺ and Ca²⁺ activation. Addition of Cl⁻ and K⁺ secretagogues altered Po of 8Kᵣ. Forskolin or carbachol (10 μM) activated the small-conductance 8Kᵣ in quiescent patches and increased Po in low-activity patches. K⁺ secretagogues, either epinephrine (5 μM) or prostaglandin E₂ (100 nM), decreased Po of 8Kᵣ in active patches. This 8Kᵣ may be involved in electrogenic secretion of Cl⁻ and K⁺ across the colonic epithelium, which requires a large basolateral membrane K⁺ conductance during maximal Cl⁻ secretion and, presumably, a lower K⁺ conductance during primary electrogenic K⁺ secretion.

Active secretion of ions across colonic epithelia serves to produce a driving force for fluid secretion and to modify the composition of secreted fluid (7, 15). Excessive rates of secretion occur in pathophysiological states such as secretory diarrhea and ulcerative colitis. As in other fluid-secreting epithelia, electrogenic Cl⁻ secretion is a major mechanism for producing fluid flow (14). Stimulating Cl⁻ secretion requires exit of K⁺ entering via the Na⁺⁻K⁺ pump and Na⁺⁻K⁺⁻2Cl⁻ co-transport, which can occur via basolateral membrane K⁺ channels (14). In mammalian colon, K⁺ secretion is stimulated together with Cl⁻ secretion, contributing to the relatively high luminal K⁺ concentration. Localization studies support the presence of K⁺ and Cl⁻ secretory capacity in columnar cells of colonic crypts (18, 19). The cellular mechanism for K⁺ secretion is electrogenic and related to the mechanism for Cl⁻ secretion. A key feature is that electrogenic K⁺ secretion measured in vitro is entirely bumetanide sensitive, suggesting an absolute requirement for Na⁺⁻K⁺⁻2Cl⁻ co-transport (15, 41). In addition, apical and basolateral membrane K⁺ channels allow exit of K⁺ from the cell. Because the rate of K⁺ secretion can vary relative to that of Cl⁻ secretion, colonic secretory cells may control K⁺ secretion, in part, by modulating basolateral K⁺ channel activity to alter the amount of K⁺ exiting into the lumen.

Activity of K⁺ channels has been detected in colonic crypts (55), a site for fluid and mucus secretion (7, 16). Channels have been observed both in the crypt base (4, 5, 39) among the first progeny of the crypt stem cell and in the tubular portion of the crypt (32, 44, 45) among the rapidly dividing cells. The predominant cell types of the crypt are columnar cells and goblet cells (16, 19). Goblet cells are distinguished from columnar cells by dense apically located mucin granules that are released during cholinergic stimulation. Cl⁻ secretion occurs with either cholinergic activation that increases intracellular Ca²⁺ or secretagogues such as vasoactive intestinal peptide and prostaglandin E₂ (PGE₂) that increase intracellular cAMP (7, 15). An increase in K⁺ conductance would serve to maintain Cl⁻ secretion by allowing K⁺ exit and by developing a cell negative membrane electrical potential difference to drive conductive Cl⁻ exit through the apical membrane into the lumen. Three major types of K⁺ channels have been observed during secretory activation of isolated colonic crypts as well as colonic tumor cells such as T84 and HT29 (55): large-conductance, Ca²⁺-activated K⁺ channels (slo or BK); intermediate-conductance, Ca²⁺-activated K⁺ channels (IK₁); small-conductance, cAMP-activated K⁺ channels (KvLQT/minK). These K⁺ channels belong to the greater group of K⁺ channel proteins that have similar pore-forming domains but distinct regulatory domains and components (10, 27).
Guinea pig distal colon can produce high rates of K\(^+\) and Cl\(^-\) secretion in response to secretagogues (17, 41). Both epinephrine and low concentrations of PGE\(_2\) stimulate electrogenic K\(^+\) secretion in the absence of accompanying Cl\(^-\) secretion, whereas high concentrations of PGE\(_2\) or forskolin stimulate electrogenic secretion of both K\(^+\) and Cl\(^-\). Thus guinea pig distal colon provides a comparison between these two modes of secretion so that the varied roles of basolateral K\(^+\) channels can be examined. In particular, increased basolateral membrane K\(^+\) channel activity would aid Cl\(^-\) secretion by enhancing conductive Cl\(^-\) exit, whereas decreased activity would increase K\(^+\) secretion by limiting exit of K\(^+\) into the interstitial space. The present study has indicated that cells in colonic crypts exhibit an inwardly rectified K\(^+\) channel, \(\delta K_A\), that has characteristics distinct from other K\(^+\) channels previously observed in colonic epithelia. Both cAMP- and Ca\(^{2+}\)-dependent secretagogues activate \(\delta K_A\), whereas K\(^+\) secretagogues moderate channel activity to lower levels.

**METHODS**

Male guinea pigs (400–650 g body wt) received standard guinea pig chow and water ad libitum. Guinea pigs were killed by decapitation in accordance with a protocol approved by the Wright State University Institutional Laboratory Animal Care and Use Committee. Distal colon was removed and defined as the ~20-cm-long segment ending roughly 5 cm from the rectum. Colonic segments were cut open along the mesenteric line and flushed with ice-cold Ringer solution to remove fecal pellets. Epithelium was separated from underlying submucosa and muscle layers by using a glass slide to gently scrape along the length of the colonic segment. The plane of dissection occurred at the base of the crypts such that only components of the mucosa immediately adherent to the epithelium remained. Portions of mucosa were mounted, with the use of cyanoacrylate glue, on to Lucite holders with apertures 1 cm wide and 4 cm long. Mucosal portions on holders were incubated at 38°C in HEPES-buffered solution with indomethacin (1 \(\mu M\)) to reduce spontaneous channel activity. Isolated crypts were stored on ice or in the refrigerator for up to 36 h.

Isolated crypts were transferred onto a polyllysine-coated plastic coverslip in the electrical recording chamber mounted on the stage of an inverted microscope (Diaphot; Nikon, Tokyo, Japan). Bathing solutions were perfused into the chamber by a peristaltic pump (Gilson, Middleton, WI) at room temperature. Pipettes were fabricated from 7052 glass (WPI, Sarasota, FL) by using a two-stage puller (Narishige, Tokyo, Japan), coated with Q-dope (GC Electronics, Rock- ford, IL), and fire-polished. Pipettes filled with either high-Na\(^+\) or high-K\(^+\) solution (Table 1, Table 2) had resistances of 5–10 MΩ and were connected to the head stage of an EPC-7 patch-clamp amplifier (List Medical, Darmstadt, Germany) via a 150 mM KAC agar salt bridge inside a holder containing a Ag-AgCl electrode (12). The reference electrode was a Ag-AgCl pipette connected to the bath through a 150 mM KCl agar salt bridge. Currents were recorded on videotape at 3-kHz filtering with a pulse code-modulated videocassette recorder (Vetter Instruments, Rebersburg, PA). Seals were made on the central tubular portion of isolated crypts bathed in standard HEPES-buffered Ringer solution. Seals of >1 MΩ were obtained in about one of five attempts. Occasionally, cell depolarization was produced with a high-K\(^+\) bath made by substituting 135 mM K\(^+\) for Na\(^+\) in the standard Ringer solution. Before patches were excised, the bath solution was changed to one containing EGTA (Table 1) to maintain low free Ca\(^{2+}\) (~10 \(\mu M\)) that would mimic intracellular conditions. Lower levels of bath free Ca\(^{2+}\) (~100 nM and ~10 mM free Ca\(^{2+}\)) were produced by adding only 0.1 mM or 0 mM Ca\(^{2+}\), respectively, to these bath solutions. Bath solution pH was adjusted by titration with NaOH or HCl. Solution osmolality was 292 mosM (290–294 mosM), except for the 300 KCl bath.

Drugs were added in small volumes from concentrated stock solutions. PGE\(_2\) was obtained from Cayman Chemical (Ann Arbor, MI), and epinephrine was from Elkins-Sinn (Cherry Hill, NJ). All other chemicals were obtained from Sigma Chemical (St. Louis, MO). PGE\(_2\) was prepared in an ethanol stock solution that added 0.1% ethanol at 10 \(\mu M\) PGE\(_2\); additions of 1% ethanol alone did not alter transepithelial measures of K\(^+\) or Cl\(^-\) secretion (17).

**Table 1. Patch-clamp solutions**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Na(^+)</th>
<th>K(^+)</th>
<th>Mg(^2+)</th>
<th>Ca(^{2+})</th>
<th>Cl(^-)</th>
<th>Gluconate(^-)</th>
<th>EGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-Na(^+) pipette</td>
<td>153</td>
<td>5</td>
<td>1.2</td>
<td>2.0</td>
<td>162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-K(^+) pipette</td>
<td>18</td>
<td>140</td>
<td>1.2</td>
<td>2.0</td>
<td>162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-glucuronate bath</td>
<td>14.5</td>
<td>143</td>
<td>5.0</td>
<td>1.0</td>
<td>50</td>
<td>114</td>
<td>1.0</td>
</tr>
<tr>
<td>Na-glucuronate bath</td>
<td>157.5</td>
<td>0</td>
<td>5.0</td>
<td>1.0</td>
<td>50</td>
<td>114</td>
<td>1.0</td>
</tr>
<tr>
<td>70 mM K(^+)–85 mM Na(^+) bath</td>
<td>85</td>
<td>70</td>
<td>5.0</td>
<td>1.0</td>
<td>161.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>300 mM KCl bath</td>
<td>5.5</td>
<td>300</td>
<td>5.0</td>
<td>1.0</td>
<td>312</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>RbCl bath</td>
<td>15</td>
<td>140Rb(^+)</td>
<td>5.0</td>
<td>1.0</td>
<td>161.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

All solutions contained HEPES (5 mM) and were titrated to pH 7.4 for pipette and pH 7.2 for bath solutions.

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Current data were transferred via DigiData-1200 interface to a computer for analysis using pCLAMP6 software (Axon Instruments, Foster City, CA). Currents were filtered at 700 Hz. Junction potentials at pipette tip and bath reference bridge were calculated to correct holding voltages (1, 38). Relative ion permeabilities were calculated by using the Goldman-Hodgkin-Katz potential equation together with the measured reversal potential and solution ion composition. Open probability \( P_o \) was calculated from all-points histograms of current amplitude. Area under each current peak \( A \) was determined by a Gaussian fit. \( P_o \) was obtained from the relation 
\[
P_o = \sqrt{\frac{\sum i A_i^2}{\sum A_i}}/N,
\]
with \( i \) indicating each peak starting at 0 for the baseline and increasing to \( N \), the number of active channels. A current-voltage relation was constructed from the lowest current peaks to assure that the lowest peak at each voltage indicated the closed state. Records of sufficient length (5–10 min) were obtained for each stimulatory condition to allow a reliable measure of \( N \) from the number of observed peaks (22). Currents recorded at holding potentials (\( V_{\text{hold}} \)) more positive than about \( -10 \) mV were generally too small to allow accurate measurement of \( P_o \). Kinetic analysis was performed on records containing only one channel by producing histograms of open and closed durations from an events list. For this analysis, current records were sampled at a rate of 20 \( \mu \)s/point and then filtered at 1 kHz (Gaussian) to minimize noise but also to maximize bandwidth. Log binning was used to improve fitting and display of exponential curves (24); maximal likelihood estimates were used to obtain time constants from open and closed times. Results are reported as means \( \pm \) SE. Statistical comparisons were made by using two-tailed Student’s \( t \)-test for paired comparisons, with significant difference accepted at \( P < 0.05 \).

**RESULTS**

Basolateral membranes of isolated crypts were readily accessible to sealing with patch pipettes; all results were from seals on the middle (tubular) section of isolated crypts. Differences between columnar and goblet cells could not be discerned readily to identify the cell type recorded. Spontaneous single-channel currents were observed while cell-attached consistent with \( K^+ \) channel activity (Fig. 1), with both high-\( Na^+ \) and high-\( K^+ \) pipette solutions (Table 1). The reversal potential in cell-attached recordings supported identification of channel types producing currents. For crypt epithelial cells (19, 31), currents from \( K^+ \) and \( Cl^- \) channel currents in crypt cells. Current traces are shown for \( K^+ \) channels from cell-attached patches of basolateral membrane in isolated colonic crypts. Closed states are indicated by dashed lines. \( A \): currents at several holding potentials \( V_{\text{hold}} \) are shown for an \(-9\)-pS \( K^+ \) channel with high-\( Na^+ \) pipette solution, containing 5 mM \( K^+ \) (see Table 1). \( B \): currents for an inwardly rectified \(-9\)-pS \( K^+ \) channel with high-\( K^+ \) channel activity (Fig. 1), with both high-\( Na^+ \) and high-\( K^+ \) pipette solutions (Table 1). The reversal potential in cell-attached recordings supported identification of channel types producing currents. For crypt epithelial cells (19, 31), currents from \( K^+ \) and \( Cl^- \)
channels recorded with high-Na\(^+\) pipette solution would be expected to reverse at negative and positive \(V_{\text{hold}}\), respectively, as determined by the ion concentration gradients. Thus, at resting membrane electrical potential difference (\(V_{\text{hold}} = 0\) mV), K\(^+\) currents would be outward and Cl\(^-\) currents would be inward (net outward Cl\(^-\) flow). In addition, nonselective cation channel currents would reverse at large positive \(V_{\text{hold}}\), corresponding to a cell membrane electrical potential difference of 0 mV. Use of high-K\(^+\) pipette solution had the advantage of increasing the size of inward K\(^+\) currents, thus permitting better detection of small-conductance channels. Because the equilibrium potential for K\(^+\) would be near a membrane electrical potential difference of 0 mV with roughly equal K\(^+\) concentrations inside and out, cell-attached reversal potentials of K\(^+\) channel currents with high-K\(^+\) pipette solution allowed a rough estimate of cell membrane electrical potential difference.

Currents consistent with Cl\(^-\) channels, as distinguished by reversal potentials, also were observed (30). Nonselective cation channels (52) reversing at positive \(V_{\text{hold}}\) were not observed in cell-attached patches. Rarely (5 of 304 patches, 1.6%), nonselective cation channels were observed reversing at \(V_{\text{hold}}\) of 0 mV, suggesting a depolarization of membrane electrical potential difference for these cells. Changing the bath solution to a high-K\(^+\) solution, which presumably depolarized cells, resulted occasionally (9 of 57 patches, 16\%) in the appearance of nonselective cation channels in cell-attached patches. For patches with nonselective cation channels, two to six channels were present with a voltage-independent single-channel conductance of \(\sim 25\) pS.

The most common K\(^+\) channel activity with high-Na\(^+\) pipette solution (10 of 73 patches, 14\%) had a linear current-voltage relation with a single-channel conductance (\(\gamma\)) of 9 pS (Figs. 1A and 2). During recording with high-K\(^+\) pipette solution, inwardly rectified current-voltage relations were observed (58 of 231 patches, 25\%) with \(\gamma\) of 9 and 19 pS at \(V_{\text{hold}} = 0\) mV (Figs. 1, B and C, and 2). These two channel behaviors with high-K\(^+\) pipette solution may be specific conductance states of the same channel, because they were observed together (3 of 6 patches with large-conductance \(\gamma\) channels, 50\%). Cell-attached currents consistent with a K\(^+\) channel of \(\sim 85\) pS also were seen in one patch with the standard bath solution (Figs. 1D and 2A) and in one other patch with high-K\(^+\) bath solution that presumably depolarized cells. Apparent higher incidence of K\(^+\) channels with high-K\(^+\) pipette solution may only reflect the generally greater ease of identifying the resulting larger inward K\(^+\) currents.

Ion selectivity of \(\gamma\)K\(_{\text{ir}}\). Activity of \(\gamma\)K\(_{\text{ir}}\) generally persisted after excision into an inside-out (I/O) configuration (17 of 18 patches, 94\%), which allowed ion selectivity to be determined more precisely. With a high-K\(^+\) pipette solution and a K-gluconate bath solu-

Fig. 2. K\(^+\) channel conductive properties. A: current-voltage relations are shown for 4 types of K\(^+\) channel activity observed in cell-attached patches of crypt basolateral membrane (means ± SE). With high-Na\(^+\) pipette solution, single-channel currents were linearly dependent on voltage (○, \(n = 6\)). With high-K\(^+\) pipette solution, currents were inwardly rectified, and those exhibiting both inward and outward currents were averaged (●, \(n = 15\)). Larger inwardly rectified currents forming a distinct group were averaged (▲, \(n = 6\)). A larger conductance (\(\sim 85\) pS) K\(^+\) channel recorded with high-K\(^+\) pipette solution also is shown (●, \(n = 1\)). Symbols often obscure small error bars. I, current. B: single-channel conductance (chord conductance, \(\gamma\)) is shown from current-voltage relations in A.
tion (containing 50 mM Cl\(^{-}\), Table 1), the reversal potential was near 0 mV (Fig. 3A), as expected for a cation channel. Increasing bath solution K\(^{+}\) concentration shifted the reversal potential to negative voltages (Fig. 3A) consistent with cation selectivity. Relative anion permeability (P\(_{\text{Cl}}\)/P\(_{\text{K}}\)) was <0.03 (n = 9). Lowering bath solution K\(^{+}\) concentration by substitution with Na\(^{+}\) shifted the reversal potential to positive voltages, as expected for a K\(^{+}\)-selective channel (Fig. 3A). Relative Na\(^{+}\) permeability (P\(_{\text{Na}}\)/P\(_{\text{K}}\)) was 0.02 ± 0.02 (n = 6). Substituting bath K\(^{+}\) completely with Na\(^{+}\) produced steep inwardly rectified currents with- out outward currents (Fig. 3C), also consistent with high selectivity for K\(^{+}\) over Na\(^{+}\). Similarly (Fig. 3A), relative Rb\(^{+}\) permeability (P\(_{\text{Rb}}\)/P\(_{\text{K}}\)) was 1.12 (n = 2, range 1.05–1.19). Currents from the 9-pS channel with high-Na\(^{+}\) pipette and Na-gluconate bath solution also were completely inwardly rectified (Fig. 3C), supporting identification as a K\(^{+}\) channel. Ion selectivity of the 19-pS \(^{\text{sp}}\)K\(_{ir}\) also supported high preference for K\(^{+}\) over Na\(^{+}\) or Cl\(^{-}\) (data not shown). These current measurements indicate that the observed channels were K\(^{+}\) selective with significant Rb\(^{+}\) permeability, suggesting a divergence from other inwardly rectified K\(^{+}\) channels (8, 49).

**Concentration dependence of \(^{\text{sp}}\)K\(_{ir}\).** Increasing K\(^{+}\) concentration at the cytoplasmic face of the patch (0, 70, and 143 mM; with constant ionic strength) increased \(\gamma\) of \(^{\text{sp}}\)K\(_{ir}\) at positive membrane potentials (V\(_{m}\)) (Fig. 3D), consistent with saturation of outward flow at <70 mM K\(^{+}\). Further increase of K\(^{+}\) concentration to 300 mM (with increased ionic strength) led to ~45% larger \(\gamma\) at positive V\(_{m}\) (Fig. 3B). For inward K\(^{+}\) flow from the pipette, decreasing K\(^{+}\) concentration in the bath (with constant ionic strength) did not alter \(\gamma\) at large negative V\(_{m}\) (Fig. 3D), but high bath K\(^{+}\) concentration (300 mM; with higher ionic strength) increased \(\gamma\) at negative V\(_{m}\) (Fig. 3B). One possible explanation for this apparent influence of cytoplasmic K\(^{+}\) on K\(^{+}\) influx, and efflux, is that the small-conductance state of \(^{\text{sp}}\)K\(_{ir}\) was converted to the large-conductance state by increased ionic strength, since \(\gamma\) at negative V\(_{m}\) with 300 mM bath K\(^{+}\) (Fig. 3B) was similar to \(\gamma\) of the large-conductance \(^{\text{sp}}\)K\(_{ir}\) (Fig. 2B).

During cell-attached recording with high-Na\(^{+}\) pipette solutions (Fig. 2), outward rectification was not apparent for the 9-pS K\(^{+}\) channel even though pipette K\(^{+}\) concentration was only 5 mM (Table 1). Lack of outward rectification with an outwardly directed concentration gradient suggests that this K\(^{+}\) channel actually was an inward rectifier, similar to previous observations on colonic K\(^{+}\) channels (39). Relative Rb\(^{+}\) conductance of \(^{\text{sp}}\)K\(_{ir}\) was similar to that with K\(^{+}\) (Fig. 3B), indicating that the mechanism producing rectification (27) apparently was not altered appreciably by Rb\(^{+}\) substitution for K\(^{+}\) at the cytoplasmic face of the channel. Similarity of K\(^{+}\) efflux with high-Na\(^{+}\) and high-K\(^{+}\) pipette solutions, as indicated by \(\gamma\) at positive V\(_{\text{hold}}\) (Fig. 2B), supports the suggestion that these two K\(^{+}\) channel activities (Fig. 1, A and B) were an identical channel type, an inward rectifier.

Voltage dependence of \(\gamma\) for \(^{\text{sp}}\)K\(_{ir}\). Excision into an I/O configuration can lead to altered channel behavior as cytoplasmic components are lost. Single-channel conductance of \(^{\text{sp}}\)K\(_{ir}\) was not changed dramatically by excision into a high-K\(^{+}\) solution that mimicked intracellular ion composition (Figs. 2B and 3B). Generally the small-conductance state was present, although the large-conductance state was observed (2 of 18 patches, 11%). However, a direct comparison of conductance-voltage dependence for cell-attached and excised conditions requires an estimate of resting cell membrane electrical potential difference (V\(_{\text{cell}}\)). Assuming that cell-attached reversal potentials of K\(^{+}\) channels with high-K\(^{+}\) pipette solution represented a V\(_{\text{cell}}\) of 0 mV, cell-attached V\(_{\text{hold}}\) then can be adjusted to indicate actual V\(_{m}\) (Fig. 3E). Excised \(\gamma\) appeared to conform best in size to the small-conductance state seen with cell-attached recordings. The ~50 mV rightward shift in voltage dependence for \(\gamma\) indicates that cytoplasmic factors controlling \(\gamma\) may have been lost upon excision. One possibility is that Mg\(^{2+}\) in the bath solution (Table 1) during I/O conditions blocks \(^{\text{sp}}\)K\(_{ir}\) with different voltage dependence than the native cytoplasmic components (27).

**Kinetic modes of \(^{\text{sp}}\)K\(_{ir}\).** P\(_{o}\) of \(^{\text{sp}}\)K\(_{ir}\) during cell-attached recording was voltage independent but occurred in two distinct modes (Fig. 4A), moderate activity (P\(_{o}\) = 0.41 ± 0.01, n = 8) and low activity (P\(_{o}\) = 0.09 ± 0.01, n = 6). Abrupt transitions between low and moderate states were observed (4 of 58 patches with \(^{\text{sp}}\)K\(_{ir}\), 7%) but were not reversed, suggesting that a voltage-independent regulatory process controlled transitions between these two kinetic modes of \(^{\text{sp}}\)K\(_{ir}\). Excision into an I/O configuration increased P\(_{o}\) of low-activity \(^{\text{sp}}\)K\(_{ir}\), producing a kinetic mode with brief open and closed events (Fig. 4B); transition to moderate P\(_{o}\) occasionally occurred only several minutes after excision. In the excised I/O condition, P\(_{o}\) was similar to the moderate-activity state (P\(_{o}\) = 0.42 ± 0.01, n = 5) regardless of whether cell-attached activity had been low or moderate (Fig. 4C), indicating that excision may remove a cytoplasmic component that acts to limit P\(_{o}\).

Secretagogue modulation of \(^{\text{sp}}\)K\(_{ir}\) activity. Numerous secretagogues stimulate electrogenic K\(^{+}\) and Cl\(^{-}\) secretion across distal colonic epithelia (7, 15). Electrogenic K\(^{+}\) secretion is stimulated by epinephrine or PGE\(_{2}\), and at higher concentrations PGE\(_{2}\) stimulates Cl\(^{-}\) and K\(^{+}\) secretion (17, 41). In addition, the cholinergic agonist carbachol (CCh) stimulates Cl\(^{-}\) secretion. Forskolin, which increases intracellular cAMP through activation of adenylate cyclase, also stimulates electrogenic Cl\(^{-}\) secretion. Spontaneous activity of \(^{\text{sp}}\)K\(_{ir}\) was observed with both high-Na\(^{+}\) (7 of 10 patches with \(^{\text{sp}}\)K\(_{ir}\), 70%) and high-K\(^{+}\) pipettes (42 of 58 patches with \(^{\text{sp}}\)K\(_{ir}\), 72%). Addition of forskolin to the bath during cell-attached recording increased both the number of open \(^{\text{sp}}\)K\(_{ir}\) (N) and apparent P\(_{o}\) in quiescent patches (Figs. 5A and 7A) and in patches with spontaneous activity (Figs. 6A and 7B). CCh added to the bath during cell-attached recording also activated \(^{\text{sp}}\)K\(_{ir}\) in a quiescent patch (Fig. 5B).
Fig. 3. Ion selectivity of inwardly rectified K⁺ channel. Current-voltage relations are shown with various bath ion compositions
(means ± SE). A: after excision into inside-out (I/O) configuration, bath solution (see Table 1) was changed among K-glucose (●,
$n = 7$), 70 mM K⁺-85 mM Na⁺ (70K/85Na; ▲, $n = 6$), 300 mM KCl (▲, $n = 2$), and RbCl (●, $n = 2$). Pipette solution was high-K⁺.
Symbols often obscure small error bars. B: single-channel conductance is shown from current-voltage relations in A. C: only inward
currents were observed for I/O patches with Na-glucose (Na-glcn) bath solution with either high-K⁺ pipette solution (●, $n = 2$)
or high-Na⁺ pipette solution (▲, $n = 5$). Currents from A with K-glucose (●) and 70K/85Na (▲) also are shown for comparison
of variations in K⁺ and Na⁺ concentration. D: single-channel conductance is shown from current-voltage relations in C. Chordconductance $g$ for Na-glucose conditions with high-K⁺ pipette solution was calculated with a reversal potential ($E_{rev}$) of +90
mV, obtained from Na⁺/K⁺ selectivity exhibited in A; $g$ at membrane potentials ($V_m$) = 0 mV ranged over −1 pS when $E_{rev}$ changed from +80 mV to +100 mV. For high-Na⁺ pipette solution, $E_{rev}$ of +70 mV was estimated by extrapolation. $E$: single-channel conductance is shown for I/O condition with K-glucose bath (●) together with those for cell-attached conditions (● and ▲) from Fig. 2B with inclusion of apparent cell membrane electrical
potential differences ($V_{cell}$) exhibited in Fig. 2A (−40 mV): $V_m = V_{hold} + V_{cell}$. Pipette solution was high-K⁺.
Using epinephrine to produce a K⁺ secretory state decreased apparent $P_o$ of forskolin-stimulated $^{sp}$Kᵢᵢ (Figs. 5A and 7B) and of spontaneously active $^{sp}$Kᵢᵢ (Fig. 6B). PGE₂ reduced apparent $P_o$ after forskolin stimulation (Fig. 6A) and in the presence of epinephrine (Fig. 6B and 7B); forskolin addition in the presence of epinephrine and PGE₂ modestly increased apparent $P_o$, though not to basal level (Fig. 6B). In a patch with low apparent $P_o$ stimulated by forskolin (Fig. 7A), PGE₂ addition led to complete inactivation of $^{sp}$Kᵢᵢ. A patch with a single $^{sp}$Kᵢᵢ (Fig. 7B) exhibited progressive modulation of $P_o$ during cumulative addition of secretagogues, increase with forskolin, decrease with epinephrine, and further decrease with PGE₂.

Forskolin and CCh, which activate Cl⁻ secretion, generally increased $N$ and $P_o$ of $^{sp}$Kᵢᵢ, whereas epinephrine and PGE₂, which activate K⁺ secretion, generally decreased $N$ and $P_o$ (Figs. 5–7). Forskolin activated $^{sp}$Kᵢᵢ (increased $N$) in quiescent patches (10 of 80 patches, 12.5%); CCh also increased $N$ of $^{sp}$Kᵢᵢ (4 of 43 quiescent patches, 9%). PGE₂ did not activate $^{sp}$Kᵢᵢ (0 of 30 quiescent patches); epinephrine rarely activated $^{sp}$Kᵢᵢ (2 of 57 quiescent patches, 3%). These observed proportions of $^{sp}$Kᵢᵢ activation include secretagogue additions to patches that may not have contained $^{sp}$Kᵢᵢ, so that actual efficacy may have been higher. Comparisons among these secretagogue results, however, do provide a relative assessment of action on $^{sp}$Kᵢᵢ, because all patches were sampled from the same group of crypts.

Secretagogue actions also were compared for patches containing spontaneously active $^{sp}$Kᵢᵢ, which eliminated the confounding effect of blank patches. Forskolin activated additional $^{sp}$Kᵢᵢ in patches with spontaneously active $^{sp}$Kᵢᵢ (3 of 12 active patches, 25%); CCh did not activate $^{sp}$Kᵢᵢ in patches with spontaneously active $^{sp}$Kᵢᵢ (0 of 6 active patches). Both epinephrine (2 of 12 active patches, 17%) and PGE₂ (4 of 17 active patches, 23%) inactivated $^{sp}$Kᵢᵢ in patches with spontaneously active $^{sp}$Kᵢᵢ; these K⁺ secretagogues did not
activate further gpKir in active patches. Large-conductance gpKir (Figs. 1C and 2) also were activated by forskolin (4 of 6 patches with large-conductance gpKir recorded, 67%); other secretagogues did not activate this large-conductance gpKir. These proportions for secretagogue action may include some patches with every gpKir present already activated such that further increases in N were not possible. In addition, these spontaneously active patches may have been in a state that altered sensitivity to secretagogues.

Secretagogue-induced changes in Po and N for gpKir, similar to the patches shown in Figs. 5–7, are summarized in Fig. 8. Po remained independent of Vhold after addition of forskolin, CCh, epinephrine, or PGE2 (Fig. 8C). Forskolin and CCh, on average, increased Po to a level (0.38 ± 0.10, n = 8, and 0.28 ± 0.05, n = 3, respectively) consistent with the moderate-activity mode of spontaneously active patches (Fig. 4A). PGE2 and epinephrine, on average, decreased Po to a level (0.22 ± 0.04, n = 10, and 0.21 ± 0.07, n = 6, respectively) between the two activity modes of spontaneously active patches (Fig. 4A). Some of the variation in secretagogue responses may depend on the order of secretagogue addition, the cell type sealed, and the prior, unknown state of each crypt. Epinephrine once activated gpKir (N = 3, Fig. 8B) only after forskolin had failed to stimulate a quiescent patch, and Po increased only to ~0.1 (Fig. 8A), similar to the low-activity mode

Fig. 5. Secretagogue activation of gpKir. Currents from gpKir were recorded during cumulative addition of secretagogues to spontaneously quiescent patches. Activity generally was recorded at 10-mV increments of Vhold. Pipette solution was high-K+. Closed states are indicated by dashed lines. A: a cell-attached patch was sequentially treated with forskolin (1 μM) and epinephrine (5 μM). Currents are shown 10.8 min after forskolin addition for Vhold of −60 mV and 6.5 min after epinephrine addition for Vhold of −50 mV; because Po was voltage independent (see Fig. 4A), kinetic changes can be assessed from these current traces. The number of open gpKir increased to 3 with forskolin. B: a cell-attached patch was treated with carbachol (CCh; 10 μM) for Vhold of −50 mV. Time after addition of CCh is noted. The number of open gpKir increased to 7 with CCh.

Fig. 6. Secretagogue modulation of gpKir. Currents from spontaneously active gpKir were recorded during cumulative addition of secretagogues. Pipette solution was high-K+. Closed states are indicated by dashed lines. A: a cell-attached patch was sequentially treated with forskolin (10 μM) and PGE2 (10 μM) for Vhold of −50 mV. Currents are shown 13.0 min after forskolin addition and 11.7 min after PGE2 addition. The number of open gpKir increased from 1 in basal to 4 with forskolin and PGE2. B: a cell-attached patch was sequentially treated with epinephrine (5 μM), PGE2 (10 μM), and forskolin (10 μM) for Vhold of −60 mV. Currents are shown 12.8 min after epinephrine addition, 9.3 min after PGE2 addition, and 8.7 min after forskolin addition. The apparent number of open gpKir in basal was 5.
of spontaneously active patches (Fig. 4A). Forskolin produced the smallest \( P_o \) increases when in the presence of other secretagogues or with \( P_o \) near 1.0. Increasing PGE2 concentration from 100 nM to 10 \( \mu \)M did not lead to further change in \( P_o \) (from 0.27 ± 0.04 to 0.24 ± 0.07, paired difference −0.03 ± 0.04, \( n = 3 \)). \( P_o \) and \( N \) changed in a manner consistent with the expected demands of transepithelial ion secretion, increasing during Cl− secretion and decreasing during K+ secretion.

**Kinetic mechanism of \( \alpha^pK_{ir} \).** Open and closed durations of \( \alpha^pK_{ir} \) activity were examined to obtain a preliminary mechanism controlling \( P_o \) changes by secretagogues. Histograms of open durations from a patch containing a single \( \alpha^pK_{ir} \) (Fig. 7B) exhibited one peak. In the basal and forskolin condition, the peak was fit well by one exponential, and after K+ secretagogue additions, two exponentials were required for an adequate fit (Fig. 9, A–D) consistent with at least two open states for \( \alpha^pK_{ir} \). Histograms of closed durations exhibited several peaks, indicating multiple closed states for \( \alpha^pK_{ir} \) (Fig. 9, E–H). Closed durations for the basal condition were fit well by four exponentials (Fig. 9E) with widely separated time constants, indicating at least four closed states for spontaneously active \( \alpha^pK_{ir} \). Closed durations during forskolin, epinephrine, and PGE2 addition also were fit best by four exponentials (Fig. 9, F–H), indicating at least four closed states for secretagogue-stimulated conditions. Forskolin stimulation of \( P_o \) (Fig. 8A) occurred through elimination of closed events longer than ~400 ms with a small relative increase in closed events of intermediate duration (Fig. 9F). Reduction of \( P_o \) by epinephrine and PGE2 occurred through a further increase in the number of closed events of intermediate duration (Fig. 9, G and H) and a decrease of time constants for open events (Fig. 9, C and D). Activation of \( \alpha^pK_{ir} \) appears to have occurred largely by altering residence in various closed states, which can be seen qualitatively in the current records (Figs. 5–7). Modulation of \( P_o \) at intermediate levels also occurred through changes of relative residence in closed states, together with shortening of the longer open time constant.

The kinetic mechanism for \( \alpha^pK_{ir} \) was analyzed further with other patches containing only one active channel; 10 \( \alpha^pK_{ir} \) were examined from 16 fits for conditions including spontaneous activity, secretagogue stimulation, and excision. One open state was apparent in basal conditions with low \( P_o \), as judged by open-duration histograms fit by a single exponential (8.0 ± 0.6 ms, \( n = 3 \)). Spontaneously active \( \alpha^pK_{ir} \) in the medium-\( P_o \) mode (\( n = 3 \)) had open-duration histograms fit by two exponentials with time constants 0.8 ± 0.2 ms (32 ± 11%) and 2.6 ± 0.1 ms (68 ± 11%). These two time constants further support the presence of at least two open states for \( \alpha^pK_{ir} \) (Fig. 10A), a short-duration state (\( \Omega_3 \)) and a longer duration state (\( \Omega_1 \)). Fits of closed-duration histograms from various conditions suggest the presence of at least five closed states, even though many were fit well individually by four exponentials.

Closed time constants clustered into six distinct groups (Fig. 10B). All of the \( \alpha^pK_{ir} \) examined had a component with a time constant in the first group, \( C_1 \). Many \( \alpha^pK_{ir} \) (7 of 10) also had contributions in the next
three groups, C2 through C4. Most \( ^{38}\text{P} K\text{ir} \) (8 of 10) had fitted components in both C2 and C3. Together, the simultaneous presence of the four shortest components suggests that these states were distinct and not variations within fewer states. In particular, the closely spaced groups C2 and C3 occurred in six successive 10-s intervals of the record in Fig. 9G (data not shown), indicating that the time constant for a single state was not simply drifting within this range unless the change oscillated continuously on a time scale much shorter than 10 s. Group C5 appeared distinct because two \( ^{38}\text{P} K\text{ir} \) were fit well with five components, C1 through C5, and one other had both components C1 and C5. A group C6 component was observed for two \( ^{38}\text{P} K\text{ir} \) with low \( P_o \), one in combination with C4 and C5. Thus, although secretagogue-induced changing of rates for entering and exiting a state could blur groupings of time constants, the minimum number of closed states for \( ^{38}\text{P} K\text{ir} \) was five, but possibly six.

Burst analysis supported the presence of two open states. Bursts were delimited by closed durations longer than 1 ms. This duration was chosen to ignore most of the closures due to the shortest apparent closed state, C1. All of the \( ^{38}\text{P} K\text{ir} \) with spontaneous activity had

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**Fig. 8.** Secretagogue dependence of \( P_o \) for \( ^{38}\text{P} K\text{ir} \). \( P_o \) and number of active channels (\( N \)) for \( ^{38}\text{P} K\text{ir} \) (12 patches) were determined from current records (see METHODS). Values for \( P_o \) and \( N \) before and during each secretagogue treatment are connected and plotted in pairs for comparison: solid lines connect changes from patches that had spontaneous activity, and dashed lines connect changes from patches that were quiescent spontaneously. A: secretagogue-induced changes of \( P_o \) are shown for previously untreated patches: forskolin (\( \bullet \), f), CCh (\( \bullet \), C), PGE2 (\( \bullet \), P), and epinephrine (\( \bullet \), e). Subsequent secretagogue responses of these treated patches also are shown (open symbols), with a label at the “before” value indicating the order of prior cumulative secretagogue additions. Average \( P_o \) for each patch was calculated from determinations at several \( V_{\text{hold}} \) (means ± SE), because \( P_o \) was voltage independent (C). Symbols often obscure small error bars. \*Significant changes (\( P < 0.05 \)). B: secretagogue-induced changes of \( N \) are shown for experiments in A. Counting current levels provides an accurate measure of \( N \) for values of 4 or smaller (20). \( N \) was >4 in only two patches, but both had \( P_o \) of 0.4 so that \( N \) probably was not seriously underestimated. C: dependence of \( P_o \) on \( V_{\text{hold}} \) is shown for the cell-attached condition. Activity was averaged for periods after addition of each secretagogue (means ± SE): forskolin (\( \bullet \), \( n = 6 \)), CCh (\( \bullet \), \( n = 3 \)), PGE2 (\( \bullet \), \( n = 9 \)), and epinephrine (\( \bullet \), \( n = 5 \)); averages include those experiments that had \( P_o \) measured for 3 or more \( V_{\text{hold}} \).
Because renal epithelial K
proportion of closures in C2 and increasing the propor-
tion of open state, OS (Fig. 9
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inwardly recti
sures in C2 and C3. For another gpKir, PGE2 reduced
roughly equal and relatively large proportion of clo-
sation free Ca2
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forskolin stimulation (Fig. 9,

The lumen negative transepithelial electrical potential
osmotic gradient that drives

Active ion secretion across epithelia produces an
osmotic gradient that drives fluid secretion (14, 15).
The lumen negative transepithelial electrical potential
difference produced by Cl
secretion results from a

burst-duration histograms fit by two exponentials (Fig.
10A). Prolongation of the longer bursts in the low-$P_0$
mode compared with the medium-$P_0$ mode was consis-
tent with the relative paucity of closures longer than 1
ms (Fig. 10B) such that burst termination is delayed
substantially. Distributions of open durations during
bursts were similar to all openings (data not shown).
Presence of the brief burst mode (0.9–1.2 ms) during
spontaneous low $P_0$ activity (Fig. 10A) suggests that a
low proportion of events (<5%) occurred in the short
open state, $O_0$ (Fig. 9A).

Excision led to increased $P_o$ of $\text{apoK}_i$ similar to the
medium-$P_o$ spontaneous activity mode (Fig. 4). Open
time constants for the excised condition (1.1 ± 0.2 ms,
24 ± 5%, and 3.5 ± 0.8 ms, 76 ± 5%, n = 3) were
indistinguishable from those in the medium-$P_o$ mode
but significantly shorter ($P < 0.05$) than that for the
low-$P_o$ mode (Fig. 10A). The distribution of closures for
the excised condition and medium-$P_o$ mode differed
from the low-$P_o$ mode by a decreased proportion of
events in C1, an increase in C2, and a lack of events in
C5 and C6 (Fig. 10B). Excision appeared to have pro-
duced an increase in $P_o$ by recreating the kinetic
conditions of the spontaneous medium-$P_o$ mode.

Reductions in $P_o$ by epinephrine and PGE2 after
forskolin stimulation (Fig. 9, G and H) occurred with a
roughly equal and relatively large proportion of clo-
sures in C2 and C3. For another $\text{apoK}_i$, PGE2 reduced $P_o$
from a spontaneous medium-$P_o$ mode by decreasing the
proportion of closures in C2 and increasing the propor-
tion in C3 (data not shown) such that the final distri-
bution was similar to that during forskolin/PGE2 stim-
ulation (Figs. 9H and 10B). Epinephrine and PGE2
appeared to decrease $P_o$ largely through a shift of
closed events into C2 and C3 as well as a relative shift
of open events into $O_S$.

**Cytoplasmic regulators.** Distinctions between known
inwardly rectified K+ channels can be made, in part,
from sensitivities to solution composition at the cyto-
plasmic face of the channel. Notably, dependence on
Ca2++ and pH can be used to aid in distinguishing
among intermediate-conductance Ca2+-activated K+
channels (IK1; KCNN4), inward rectifier K+ channels
(Kir; KCNJ), and so-called background K+ channels
(TWIK; KCNK1) (10). Activity of $\text{apoK}_i$ was similar with
bath pH of 7.2 and 6.6 (Fig. 11A), whereas increasing
bath pH to 8.1 reduced $\text{apoK}_i$ activity modestly (n = 3).
Because renal epithelial K+ channel (ROMK, Kir1.1;
KCNNJ) activity decreases with acidification (9, 36),
another channel type apparently was responsible for
this $\text{apoK}_i$ observed in crypts. Reduction in bath solu-
tion free Ca2+ (Fig. 11B) did not lead to lower activity
for $\text{apoK}_i$ (n = 4), indicating that IK1 (13, 23) alone
could not be responsible for producing these currents.

**DISCUSSION**

Active ion secretion across epithelia produces an
osmotic gradient that drives fluid secretion (14, 15).

The lumen negative transepithelial electrical potential
difference produced by Cl
secretion results from a

cellular mechanism employing apical membrane Cl
channels and basolateral membrane K+ channels. To-
gether with the K+ concentration gradient developed
by operation of the Na+-K+ pump, basolateral mem-
brane K+ conductance serves to generate a cell nega-
tive basolateral membrane electrical potential differ-
ence ($V_b$). Apical membrane Cl– channels allow Cl–
exit into the lumen, driven by the electrochemical gra-
dient. This gradient is directed outward because of the
influence of basolateral membrane K+ channels on
apical membrane electrical potential difference ($V_a$).
Continued Cl– secretion, and thus fluid secretion, de-
pends on a basolateral membrane K+ conductance
large enough to maintain Cl– exit across the apical
membrane by ensuring that $V_a$ exceeds the size of the
inwardly directed concentration gradient.

Colonic Cl– secretion in mammals is accompanied by
electrogenic K+ secretion, apparently by inclusion of
apical membrane K+ channels in Cl– secretory cells
(15, 41). For colonic secretory cells, therefore, K+ chan-
nels in both apical and basolateral membranes contrib-
ute to maintaining a cell negative $V_a$ that promotes Cl–
secretion. In addition, K+ conductance of apical mem-
brane relative to basolateral membrane contributes to
determining the proportion of intracellular K+ exiting
into the lumen. Elevated luminal K+ concentration
(19) would lower the K+ concentration gradient across
the apical membrane and thereby reduce the ability of
apical K+ channels to ensure conductive Cl– exit. Re-
lative rates of Cl– and K+ secretion vary among mam-
malian species (15), but in guinea pig distal colon these
electrogenic flows are roughly equal when stimulated
by high concentrations (>100 nM) of PGE2 (17, 41).
Activation by epinephrine (15, 41) or low concentra-
tions (<100 nM) of PGE2 (17, 41) produces electrogenic
K+ secretion without accompanying Cl– secretion. Cl–
entering via basolateral membrane Na+-K+–2Cl–
cotransporters during this type of sustained electrogene-
ic K+ secretion apparently exits across the basolateral
membrane through Cl– channels (15, 30, 41). Control
of basolateral K+ channels during primary electrogene-
ic K+ secretion, thus, also would contribute to main-
taining a driving force for Cl– exit from the cell as well as
to determining the proportion of K+ exiting into the
lumen.

**K+ channel types.** Several classes of K+ channels
have been identified by amino acid sequence homology
(10, 27). Although all seem to conserve a general pore-
forming component, wide variation occurs in other por-
tions of the sequence that presumably control channel
activation and kinetic behavior. Three of these K+
channel types exhibit distinct inwardly rectified γ Kir
(KCNJ), IK1 (KCNN4), and TWIK (KCNK1) (10). With
symmetrical 150 mM K+ concentration, γ at Vm = 0
mV is 30, 16, and 28 pS for Kir1.1 (9), IK1 (23, 25), and
TWIK (29), respectively. Specific sensitivities to pH,
Ca2++, and ATP also contribute to a functional identi-
cation of these channel types (10). In particular, the
ROMK channel (Kir1.1; KCNNJ) is inhibited by acidifi-
cation at the cytoplasmic side of the channel (9, 36).
Increasing cytoplasmic ATP also inactivates ROMK
(Kir1.1; KCNJ1) (9, 36); association with a regulatory subunit, the sulfonylurea receptor, confers even greater ATP sensitivity to Kir6 (10). Activation by cytoplasmic Ca\(^{2+}\)/H\(_{1001}\) is a key feature of IK\(_1\) shared with BK (slo; KCNMA) (10). The TWIK channel has been termed a background channel because it does not have any direct dependence on pH, Ca\(^{2+}\), or \(V_m\) (10, 29).

Behavior of K\(^+\) channels in native circumstances may vary from characteristics of overexpressed versions, however, because K\(^+\) channels may exist as heteromultimeric assemblies of channel and regulatory subunits that result in divergent properties (10, 27, 43, 51).

**Colonic K\(^+\) channels.** Three major types of K\(^+\) channels have been observed in cell-attached patches on basolateral membrane of colonic and small intestinal crypts (55): large-conductance K\(^+\) channels (5, 6, 26, 32, 33, 35, 42), intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels (4, 5, 20, 35, 42, 44), and very small conductance K\(^+\) channels (57). Nonselective cation channels, with \(g\) ranging from \(\sim 100\) pS to \(>200\) pS, and some are
activated by increases in Ca\(^{2+}\) activity at the cytoplasmic face of the channel (5, 26, 32, 33, 35). Kinetic behavior and large conductance suggest that these K\(^+\) channels, and those observed in guinea pig crypts (Figs. 1D and 2), are intestinal assemblies of the BK (slo; KCNMA) channel (10). Colonic intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels often are inwardly rectified (5, 20, 39, 42) with \(g\) of 30–35 pS in symmetrical 150 mM K\(^+\) concentrations at \(V_m = 0\) mV. Inwardly rectified Ca\(^{2+}\)-activated K\(^+\) channels with \(g\) of \(\approx 20\) pS have been observed as well in the colonic tumor cell line T84 (11, 49). Identification of these K\(^+\) channels as IK1 (KCNN4) (10) is supported further by detection of mRNA for IK1 in T84 cells and colonic epithelial cells (56). Another K\(^+\) channel with very small conductance (\(\approx 4\) pS) also has been detected in colonic crypts, with the use of noise analysis (57), similar in size to KvLQT1 (KCNQ1) assembled with the regulatory subunit minK\(^+\) (KCN) (58). Identity of this crypt K\(^+\) channel as KvLQT1/minK\(^+\) (KCNQ1/ KCNE) is supported by in situ hybridization localizing mRNA for both subunits in colonic crypt cells (46).

The predominant K\(^+\) channel type found in this study of guinea pig distal colonic crypts was an inwardly rectified K\(^+\) channel, \(\delta K_{ir}\) (Fig. 1B). A larger conductance K\(^+\) channel was observed rarely (Fig. 1D) that may correspond to the BK channel (slo; KCNMA) (10). Identity of the \(\delta K_{ir}\) observed in basolateral membranes is uncertain. With physiological external K\(^+\) and Na\(^+\) concentrations (Figs. 1A and 2), \(\delta K_{ir}\) apparently had a linear current-voltage relation. A less commonly observed inwardly rectified K\(^+\) channel (Fig. 1C), which may be another conductance state of \(\delta K_{ir}\), had steeper rectification than \(\delta K_{ir}\) at negative \(V_m\) (Fig. 3E). Insensitivity to changes in Ca\(^{2+}\) activity on the cytoplasmic side of \(\delta K_{ir}\) (Fig. 10B) suggests that IK1 (KCNN4) (10) is not a candidate protein unless other associating components could modify Ca\(^{2+}\) dependence. Insensitivity to acid pH (Fig. 10A) suggests that ROMK (Kir1.1; KCNJ1) (9, 36) also is not the protein forming \(\delta K_{ir}\). The lack of these specific controlling factors for \(\delta K_{ir}\) suggests TWIK (KCNK1) (10) as a possibility, but \(\gamma\) for TWIK with symmetrical 150 mM K\(^+\) concentrations (at \(V_m = 0\) mV) is approximately twofold larger (29) than for \(\delta K_{ir}\) (Figs. 3B). Thus \(\delta K_{ir}\) does not conform easily to any of these known inwardly rectified K\(^+\) channel types.
Conduction (Fig. 3E) and activation (Fig. 11) properties for \( \text{gpKir} \) suggest that guinea pig colonic crypts exhibit a \( \text{K}^+ \) channel distinct from those already characterized in basolateral membranes for this type of epithelial cell (55). However, several commonalities can be found between \( \text{gpKir} \) and previously observed intermediate-conductance \( \text{K}^+ \) channels. ROMK has a subconductance state of \( \sim13 \text{pS} \) corresponding to a particular phosphorylated state (34). Coexpression of the \( \text{Cl}^- \) channel cystic fibrosis transmembrane conductance channel (CFTR) with \( \text{gpKir} \) may influence its gating properties.

**Fig. 10.** Open and closed states of \( \text{gpKir} \). Time constants and proportions of events for kinetic states obtained from fits of duration histograms to mixtures of exponentials were averaged from patches containing only 1 \( \text{gpKir} \) (means ± SE, range is shown for \( n=2 \)). Spontaneous cell-attached activity with low \( P_o \) (\( n=3 \)) and medium \( P_o \) (\( n=3 \)) together with excised I/O activity (\( n=3 \)) are shown. Activity during PGE\(_2\) (\( n=2 \)) and epinephrine (\( n=1 \)) treatments also is shown. A: open states of shorter (\( O_S \)) and longer (\( O_L \)) time constants clustered into groups. For those \( \text{gpKir} \) that did not exhibit a fit in a particular closed-duration group, a proportion of zero was included in the group average; \( n \) is indicated on plots for those cases. *Significantly different from medium-\( P_o \) or excised conditions (\( P<0.05 \)). Vertical dotted lines demarcate apparent ranges of observed time constants; average values (from spontaneous activity) for each group were \( O_S, 0.3 \pm 0.01 \text{ms}, n=10; O_L, 3.2 \pm 0.1 \text{ms}, n=9; C_3, 12.7 \pm 1.5 \text{ms}, n=8; C_4, 148.4 \pm 35.2 \text{ms}, n=8; C_5, 1.31 \pm 54 \text{s}, n=4; \) and \( C_6, 25.2 \pm 10.7 \text{s}, n=2. \)

Conduction (Fig. 3E) and activation (Fig. 11) properties for \( \text{gpKir} \) suggest that guinea pig colonic crypts exhibit a \( \text{K}^+ \) channel distinct from those already characterized in basolateral membranes for this type of epithelial cell (55). However, several commonalities can be found between \( \text{gpKir} \) and previously observed intermediate-conductance \( \text{K}^+ \) channels. ROMK has a subconductance state of \( \sim13 \text{pS} \) corresponding to a particular phosphorylated state (34). Coexpression of the \( \text{Cl}^- \) channel cystic fibrosis transmembrane conductance channel (CFTR) with \( \text{gpKir} \) may influence its gating properties.

**Fig. 11.** Control of \( \text{gpKir} \). Currents from \( \text{gpKir} \) were recorded in excised I/O configuration during changes in bath solution. Pipette solution was high-\( K^+ \). Closed states are indicated by dashed lines. A: currents are shown for bath solution pH (see methods) of 7.2, 8.1, or 6.6 for \( V_{\text{hold}} \) of \( -50 \text{mV} \). \( P_o \) was voltage independent: 0.39 ± 0.02 (pH 7.2), 0.39 ± 0.03 (pH 6.6), and 0.28 ± 0.03 (pH 8.1); the number of active \( \text{gpKir} \) was 4. B: currents are shown for bath solution free \( \text{Ca}^{2+} \) concentration (see METHODS) of \( \sim10 \mu\text{M} \) (basal), \( 100 \text{nM} \) (low \( \text{Ca}^{2+} \)), or \(<10 \text{nM} \) (0 \( \text{Ca}^{2+} \)) for \( V_{\text{hold}} \) of \( -50 \text{mV} \). \( P_o \) was voltage independent: 0.46 ± 0.01 (basal), 0.45 ± 0.01 (low \( \text{Ca}^{2+} \)), and 0.42 ± 0.01 (0 \( \text{Ca}^{2+} \)); the number of active \( \text{gpKir} \) was 5.
regulator with ROMK leads to inwardly rectified K⁺ channels with γ of about half (~15 pS) that for ROMK alone (43). In addition, heteromeric assembly of Kir4.1 with Kir5.1 increases γ by approximately twofold (51). Ca²⁺-dependent K⁺ channels in T84 cells have low Pₒ independent of Vₘ (50), similar to that for spKir (Fig. 4A). Other Ca²⁺-dependent K⁺ channels of intermediate conductance exhibit Pₒ that increases (5) or decreases (56) at more positive Vₘ. ROMK has high Pₒ (~0.9) that is relatively independent of Vₘ (40). High relative permeability to Rb⁺ (Fig. 3) appears to distinguish spKir from both ROMK (8) and IK1 (49). Although none of the currently established K⁺ channels (10) has identical characteristics to spKir, a distinct assembly of channel and regulatory subunits (27) presumably confers the properties observed for spKir.

**Regulation of Cl⁻ and K⁺ secretion.** Control of spKir in the basolateral membrane participates in production of Cl⁻ and K⁺ secretion by contributing to maintenance of Vₘ that drives Cl⁻ exit into the lumen while also adjusting the proportion of K⁺ exit into the lumen. Activation of spKir occurred with two Cl⁻ secretagogues, forskolin and CCh (Figs. 5–7), consistent with this channel being used to augment basolateral membrane K⁺ conductance. Both the number of active spKir (Nᵢₘ) and Pₒ increased (Fig. 8) so that basolateral membrane K⁺ conductance (gK) would increase: gK = NᵢₘPₒγK. The K⁺ secretagogues PGE₂ and epinephrine led to decreased gK primarily by reducing Pₒ (Fig. 8) and occasionally by deactivating spKir (Nᵢₘ). This type of control would suit the requirements of balancing K⁺ and Cl⁻ secretory rates by moderating basolateral membrane K⁺ conductance within a precise range.

Regulatory modes for spKir can be distinguished on the basis of the kinetics producing Pₒ. Spontaneous activity exhibited two modes, with low and moderate Pₒ (Figs. 4A and 10). The cAMP-dependent agent forskolin produced moderate Pₒ with kinetics different from the spontaneous mode (Figs. 8A, 9, and 10). The K⁺ secretagogues PGE₂ and epinephrine reduced Pₒ to an intermediate level with kinetics distinguishable from the other three modes (Figs. 8A, 9, and 10). These four kinetic modes of spKir presumably are produced by distinct regulatory mechanisms characteristic of the components making up this channel complex.

A kinetic scheme for spKir must include two open states and at least five, possibly six, closed states (Fig. 10). Although the connections for these states were not determined uniquely by the data available, several features controlling each mode were apparent. Long-duration closures in the C₃ and C₅ groups were associated only with the low-Pₒ mode. The medium-Pₒ mode also differed from the low-Pₒ mode by having similar contributions from C₁ and C₂. Intermediate Pₒ of the K⁺ secretory mode occurred with a relatively high contribution from C₃. Both the low-Pₒ and forskolin modes had open durations dominated by a single state with a time constant approximately four times longer than Oₙ of the medium-Pₒ and K⁺ secretory modes.

A common feature of many K⁺ channels, including spKir, appears to be activation through reduced numbers of long-duration closures, although the mechanism producing this kinetic change is distinct for each channel type. Similar to the loss with forskolin of spKir, closed durations ranging from 0.4 to 10 s (Fig. 9, E and F), cAMP-dependent activation eliminated longer closed durations of an ATP-dependent, inwardly rectified K⁺ channel in the basolateral membrane of proximal tubules (37). Protein kinase A (PKA) together with ATP stimulates an 85-pS K⁺ channel from the lateral membrane of cortical collecting duct largely through a decreased number of the long-duration closures as well as an increased number of the long-duration openings (53). Modulation of ROMK (Kir1.1; KCNJ1) via alkalinization increases Pₒ by decreasing the number of long-duration closures without changing open durations (9, 36). Heteromeric Kir4.1-Kir5.1 (KCNJ10–KCNJ16) also increases Pₒ upon alkalinization through a loss of long-duration closures (59). Stimulation of IK1 (KCNN4) by the K⁺ channel opener 1-EBIO occurs through a decrease in long closed durations without any change in open durations (48). A small-conductance Ca²⁺-activated K⁺ channel (SK2; KCNN2) activates with increased cytoplasmic Ca²⁺, in part, by losing long duration closures (21).

Activation of K⁺ channels can result from phosphorylation via cellular kinases (54). Both the cAMP-dependent PKA and the Ca²⁺-dependent protein kinase C (PKC) have been implicated in K⁺ channel regulation (10, 27, 54). Increasing cAMP either directly or with forskolin stimulates activity of BK channels in rabbit colonic crypts (33), intermediate-conductance K⁺ channels (IK1) in human colonic crypts (44), and very small conductance K⁺ channels (KvLQT1/MinK) in rat colonic crypts (57). Loss of channel activity after excision into an I/O configuration often can be returned by adding ATP to the bathing solution or by inhibiting phosphatase activity (2), suggesting that channel Pₒ can be controlled through a balance between kinase and phosphatase activity (13, 28, 39, 50, 54, 57).

The tendency for excision to increase Pₒ of spKir (Fig. 4) suggests that a cytosolic inhibitory component was lost. One possibility could be that a membrane-resident phosphatase (2) dephosphorylated spKir at an inhibitory site, similar to the negative influence of PKC on ROMK (54). Excision activation (Fig. 4B) and forskolin activation (Figs. 6A and 7B) both increase Pₒ of spKir into the range of the spontaneous medium-Pₒ mode (Fig. 4A). In part, Pₒ increased because spKir did not enter long-lived closed states. However, during forskolin treatment spKir openings were predominantly to the longer open state (Fig. 9B), whereas after excision openings occurring to both states and Oₙ had a shorter time constant (Fig. 10A). These two modes of activation could be reconciled with a phosphorylation/dephosphorylation mechanism if at least two phosphorylation sites were to exist with opposing action on Pₒ such that relieving the negative control is sufficient for increased activity.

Cholinergic activation of spKir apparently does not proceed through direct influence of cytoplasmic Ca²⁺ on the channel (Fig. 10B), as occurs for other interme-
diate-conductance $K^+$ channels (4, 11, 13, 23, 44, 49). Instead, $Ca^{2+}$ dependence may be indirect, conferred perhaps via a $Ca^{2+}$-activated kinase such as PKC or calmodulin-dependent kinase. Reduction of $\delta P_{\text{Kr}}$, $P_o$ by the $K^+$ secretagogues PGE$_2$ and epinephrine (Fig. 8) generally did not drop to the low spontaneous levels (Fig. 4A). Rather than returning to a condition with long-duration closures, closures to states with intermediate time constants increased in number (Figs. 9 and 10). Somatostatin also reduces $P_o$ to a non-zero value for a cAMP-activated $K^+$ channel in human colonic crypts in concert with only a $\sim 14\%$ decrease of intracellular cAMP levels (45). Although PGE$_2$ and epinephrine can act through receptors that increase cytoplasmic cAMP (15), this inhibitory control of $\delta P_{\text{Kr}}$ may occur through another second messenger pathway such that several separable mechanisms may be involved in controlling $\delta P_{\text{Kr}}$ kinetics.

Colonic crypts possess several types of $K^+$ channels (55), and each may serve specific facets of the cellular requirements for $K^+$ conductance. These needs may not be identical for the two predominant cell types in the crypt, columnar and goblet. Because mucus release from columnar cells and goblet cells is controlled by distinct secretagogues (16), responsiveness to cholinergic stimulation by CCh may indicate a goblet cell regulatory mode. However, attempts to examine cell type-specific responses may have been complicated by differences in patching efficacy. Also in this study, incidences of channel appearance and activation were low enough to obscure further any cell type distinctions that might exist. However, the contribution of $\delta P_{\text{Kr}}$ to basolateral membrane $K^+$ conductance of crypt epithelium would be a 9-pS channel (Fig. 2) with a $P_o$ that can be modulated in the range from 0.1 to 0.5 (Fig. 8). For the situations of $Cl^-$ and $K^+$ secretion, this feature of subtle modulations in $P_o$ may allow secretory cells to adjust basolateral $K^+$ conductance for a precise balance in rates of $K^+$ exit and maintenance of membrane electrical potential differences ($V_a$ and $V_b$).

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