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 (gKir) 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 gKir to the medium activity mode; Po of gKir was independent of bath Ca²⁺ activity and bath acidification. Addition of Cl⁻ and K⁺ secretagogues altered Po of gKir. Forskolin or carbachol (10 μM) activated the small-conductance gKir in quiescent patches and increased Po in low-activity patches. K⁺ secretagogues, either epinephrine (5 μM) or prostaglandin E₂ (100 nM), decreased Po of gKir in active patches. This gKir 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⁻ cotransport (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, \(\delta \text{PK}_{\text{fi}}\), that has characteristics distinct from other K\(^+\) channels previously observed in colonic epithelia. Both cAMP- and Ca\(^{2+}\)-dependent secretagogues activate \(\delta \text{PK}_{\text{fi}}\), 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 killed by decapitation in accordance with a protocol approved via a 150 mM KCl agar salt bridge inside a holder containing an 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 M) that would mimic intracellular conditions. Lower levels of bath free Ca\(^{2+}\) (~100 nM and ~10 M free Ca\(^{2+}\)) were produced by adding only 0.1 mM or 0.0 M Ca\(^{2+}\), respectively, to these bath solutions. Bath solution pH was adjusted by titration with NaOH or HCl. Solution osmolarity 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 μM 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>114</td>
<td>1.0</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>114</td>
<td>1.0</td>
</tr>
<tr>
<td>K-glucosinate 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-glucosinate 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.
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

\[
P_o = \frac{\sum i A_i}{\sum i A_i} / N,
\]

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 measurement of \( N \) from the number of observed peaks (22). Currents recorded at holding potentials \( (V_{hold}) \) 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 \( Cl^- \)
channels recorded with high-Na+ pipette solution would be expected to reverse at negative and positive Vhold, respectively, as determined by the ion concentration gradients. Thus, at resting membrane electrical potential difference (Vhold = 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 Vhold, 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 Vhold were not observed in cell-attached patches. Rarely (5 of 304 patches, 1.6%), nonselective cation channels were observed reversing at Vhold 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 ∼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 (γ) 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 γ of 9 and 19 pS at Vhold = 0 mV (Figs. 1B, 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 states of the same channel, because they were observed together (3 of 6 patches with large-conductance 50%, 50%). Cell-attached currents consistent with a K+ channel of ∼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 gpKir. Activity of gpKir 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-

![Graph](image-url)
tion (containing 50 mM Cl\textsuperscript{-}, Table 1), the reversal potential was near 0 mV (Fig. 3A), as expected for a cation channel. Increasing bath solution K\textsuperscript{+} concentration shifted the reversal potential to negative voltages (Fig. 3A) consistent with cation selectivity. Relative anion permeability (P\textsubscript{Cl}/P\textsubscript{K}) was <0.03 (n = 9). Lowering bath solution K\textsuperscript{+} concentration by substitution with Na\textsuperscript{+} shifted the reversal potential to positive voltages, as expected for a K\textsuperscript{+}-selective channel (Fig. 3A). Relative Na\textsuperscript{+} permeability (P\textsubscript{Na}/P\textsubscript{K}) was 0.02 ± 0.02 (n = 6). Substituting bath K\textsuperscript{+} completely with Na\textsuperscript{+} produced steep inwardly rectified currents without outward currents (Fig. 3C), also consistent with high selectivity for K\textsuperscript{+} over Na\textsuperscript{+}. Similarly (Fig. 3A), relative Rb\textsuperscript{+} permeability (P\textsubscript{Rb}/P\textsubscript{K}) was 1.12 (n = 2, range 1.05–1.19). Currents from the 9-pS channel with high-Na\textsuperscript{+} pipette and Na-gluconate bath solution also were completely inwardly rectified (Fig. 3C), supporting identification as a K\textsuperscript{+} channel. Ion selectivity of the 19-pS \textsuperscript{85}Rb\textsuperscript{+} was also supported high preference for K\textsuperscript{+} over Na\textsuperscript{+} or Cl\textsuperscript{-} (data not shown). These current measurements indicate that the observed channels were K\textsuperscript{+} selective with significant Rb\textsuperscript{+} permeability, suggesting a divergence from other inwardly rectified K\textsuperscript{+} channels (8, 49).

**Concentration dependence of \textsuperscript{85}Rb\textsuperscript{+}.** Increasing K\textsuperscript{+} concentration at the cytoplasmic face of the patch (0, 70, and 143 mM, with constant ionic strength) increased \( \gamma \) of \textsuperscript{85}Rb\textsuperscript{+}, at positive membrane potentials (V\textsubscript{m}) (Fig. 3D), consistent with saturation of outward flow at <70 mM K\textsuperscript{+}. Further increase of K\textsuperscript{+} concentration to 300 mM (with increased ionic strength) led to a ~45% larger \( \gamma \) at positive V\textsubscript{m} (Fig. 3B). For inward K\textsuperscript{+} flow from the pipette, decreasing K\textsuperscript{+} concentration in the bath (with constant ionic strength) did not alter \( \gamma \) at large negative V\textsubscript{m} (Fig. 3D), but high bath K\textsuperscript{+} concentration (300 mM; with higher ionic strength) increased \( \gamma \) at negative V\textsubscript{m} (Fig. 3B). One possible explanation for this apparent influence of cytoplasmic K\textsuperscript{+} on K\textsuperscript{+} influx, and efflux, is that the small-conductance state of \textsuperscript{85}K\textsubscript{ir} was converted to the large-conductance state by increased ionic strength, since \( \gamma \) at negative V\textsubscript{m} with 300 mM bath K\textsuperscript{+} (Fig. 3B) was similar to \( \gamma \) of the large-conductance \textsuperscript{85}K\textsubscript{ir} (Fig. 2B).

During cell-attached recording with high-Na\textsuperscript{+} pipette solutions (Fig. 2), outward rectification was not apparent for the 9-pS K\textsuperscript{+} channel even though pipette K\textsuperscript{+} concentration was only 5 mM (Table 1). Lack of outward rectification with an outwardly directed concentration gradient suggests that this K\textsuperscript{+} channel actually was an inward rectifier, similar to previous observations on colonic K\textsuperscript{+} channels (39). Relative Rb\textsuperscript{+} conductance of \textsuperscript{85}K\textsubscript{ir} was similar to that with K\textsuperscript{+} (Fig. 3B), indicating that the mechanism producing rectification (27) apparently was not altered appreciably by Rb\textsuperscript{+} substitution for K\textsuperscript{+} at the cytoplasmic face of the channel. Similarity of K\textsuperscript{+} efflux with high-Na\textsuperscript{+} and high-K\textsuperscript{+} pipette solutions, as indicated by \( \gamma \) at positive V\textsubscript{hold} (Fig. 2B), supports the suggestion that these two K\textsuperscript{+} channel activities (Fig. 1, A and B) were an identical channel type, an inward rectifier.

**Voltage dependence of \( \gamma \) for \textsuperscript{85}K\textsubscript{ir}.** Excision into an I/O configuration can lead to altered channel behavior as cytoplasmic components are lost. Single-channel conductance of \textsuperscript{85}K\textsubscript{ir} was not changed dramatically by excision into a high-K\textsuperscript{+} 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\textsubscript{cell}). Assuming that cell-attached reversal potentials of K\textsuperscript{+} channels with high-K\textsuperscript{+} pipette solution represented a V\textsubscript{cell} of 0 mV, cell-attached V\textsubscript{hold} then can be adjusted to indicate actual V\textsubscript{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\textsuperscript{2+} in the bath solution (Table 1) during I/O conditions blocks \textsuperscript{85}K\textsubscript{ir} with different voltage dependence than the native cytoplasmic components (27).

**Kinetic modes of \textsuperscript{85}K\textsubscript{ir}.** P\textsubscript{o} of \textsuperscript{85}K\textsubscript{ir} during cell-attached recording was voltage independent but occurred in two distinct modes (Fig. 4A), moderate activity (P\textsubscript{o} = 0.41 ± 0.01, n = 8) and low activity (P\textsubscript{o} = 0.09 ± 0.01, n = 6). Abrupt transitions between low and moderate states were observed (4 of 58 patches with \textsuperscript{85}K\textsuperscript{ir}, 7%) but were not reversed, suggesting that a voltage-independent regulatory process controlled transitions between these two kinetic modes of \textsuperscript{85}K\textsubscript{ir}. Excision into an I/O configuration increased P\textsubscript{o} of low-activity \textsuperscript{85}K\textsubscript{ir}, producing a kinetic mode with brief open and closed events (Fig. 4B); transition to moderate P\textsubscript{o} occasionally occurred only several minutes after excision. In the excised I/O condition, P\textsubscript{o} was similar to the moderate-activity state (P\textsubscript{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\textsubscript{o}.

**Secretagogue modulation of \textsuperscript{85}K\textsubscript{ir} activity.** Numerous secretagogues stimulate electrogenic K\textsuperscript{+} and Cl\textsuperscript{-} secretion across distal colonic epithelia (7, 15). Electrogenic K\textsuperscript{+} secretion is stimulated by epinephrine or PGE\textsubscript{2}, and at higher concentrations PGE\textsubscript{2} stimulates Cl\textsuperscript{-} and K\textsuperscript{+} secretion (17, 41). In addition, the cholinergic agonist carbachol (CCh) stimulates Cl\textsuperscript{-} secretion. Forskolin, which increases intracellular cAMP through activation of adenylyl cyclase, also stimulates electrogenic Cl\textsuperscript{-} secretion. Spontaneous activity of \textsuperscript{85}K\textsubscript{ir} was observed with both high-Na\textsuperscript{+} (7 of 10 patches with \textsuperscript{85}K\textsubscript{ir}, 70%) and high-K\textsuperscript{+} pipettes (42 of 58 patches with \textsuperscript{85}K\textsubscript{ir}, 72%). Addition of forskolin to the bath during cell-attached recording increased both the number of open \textsuperscript{85}K\textsubscript{ir} (N) and apparent P\textsubscript{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 \textsuperscript{85}K\textsubscript{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-gluconate (○, 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-gluconate (Na-glc) bath solution with either high-K⁺ pipette solution (○, n = 2) or high-Na⁺ pipette solution (□, n = 5). Currents from A with K-gluconate (○) 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. Chord-conductance for Na-gluconate 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; γ 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-glucinate 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 \( \mathrm{gpK}_{\text{ir}} \) (Figs. 5A and 7B) and of spontaneously active \( \mathrm{gpK}_{\text{ir}} \) (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 \( \mathrm{gpK}_{\text{ir}} \). A patch with a single \( \mathrm{gpK}_{\text{ir}} \) (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 \( \mathrm{gpK}_{\text{ir}} \), whereas epinephrine and PGE₂, which activate K⁺ secretion, generally decreased \( N \) and \( P_o \) (Figs. 5–7). Forskolin activated \( \mathrm{gpK}_{\text{ir}} \) (increased \( N \)) in quiescent patches (10 of 80 patches, 12.5%); CCh also increased \( N \) of \( \mathrm{gpK}_{\text{ir}} \) (4 of 43 quiescent patches, 9%). PGE₂ did not activate \( \mathrm{gpK}_{\text{ir}} \) (0 of 30 quiescent patches); epinephrine rarely activated \( \mathrm{gpK}_{\text{ir}} \) (2 of 57 quiescent patches, 3%). These observed proportions of \( \mathrm{gpK}_{\text{ir}} \) activation include secretagogue additions to patches that may not have contained \( \mathrm{gpK}_{\text{ir}} \), so that actual efficacy may have been higher. Comparisons among these secretagogue results, however, do provide a relative assessment of action on \( \mathrm{gpK}_{\text{ir}} \), because all patches were sampled from the same group of crypts.

Secretagogue actions also were compared for patches containing spontaneously active \( \mathrm{gpK}_{\text{ir}} \), which eliminated the confounding effect of blank patches. Forskolin activated additional \( \mathrm{gpK}_{\text{ir}} \) in patches with spontaneously active \( \mathrm{gpK}_{\text{ir}} \) (3 of 12 active patches, 25%); CCh did not activate \( \mathrm{gpK}_{\text{ir}} \) in patches with spontaneously active \( \mathrm{gpK}_{\text{ir}} \) (0 of 6 active patches). Both epinephrine (2 of 12 active patches, 17%) and PGE₂ (4 of 17 active patches, 23%) inactivated \( \mathrm{gpK}_{\text{ir}} \) in patches with spontaneously active \( \mathrm{gpK}_{\text{ir}} \); 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 $P_o$ and $N$ for gpKir, similar to the patches shown in Figs. 5–7, are summarized in Fig. 8. $P_o$ remained independent of $V_{\text{hold}}$ after addition of forskolin, CCh, epinephrine, or PGE$_2$ (Fig. 8C). Forskolin and CCh, on average, increased $P_o$ to a level ($0.38 \pm 0.10, n = 8$, and $0.28 \pm 0.05, n = 3$, respectively) consistent with the moderate-activity mode of spontaneously active patches (Fig. 4A). PGE$_2$ and epinephrine, on average, decreased $P_o$ to a level ($0.22 \pm 0.04, n = 10$, and $0.21 \pm 0.07, n = 6$, 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 $P_o$ increased only to $0.1$ (Fig. 8A), similar to the low-activity mode.
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 PGE$_2$ concentration from 100 nM to 10 nM 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 $\delta K{\textit{ir}}$.** Open and closed durations of $\delta K{\textit{ir}}$ activity were examined to obtain a preliminary mechanism controlling $P_o$ changes by secretagogues. Histograms of open durations from a patch containing a single $\delta K{\textit{ir}}$ (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 $\delta K{\textit{ir}}$. Histograms of closed durations exhibited several peaks, indicating multiple closed states for $\delta K{\textit{ir}}$ (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 $\delta K{\textit{ir}}$. Closed durations during forskolin, epinephrine, and PGE$_2$ 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 PGE$_2$ 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 $\delta K{\textit{ir}}$ 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 $\delta K{\textit{ir}}$ was analyzed further with other patches containing only one active channel; 10 $\delta K{\textit{ir}}$ 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 $\delta K{\textit{ir}}$ 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 $\delta K{\textit{ir}}$ (Fig. 10A), a short-duration state (O$_3$) and a longer duration state (O$_4$). 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 $\delta K{\textit{ir}}$ examined had a component with a time constant in the first group, C$_1$. Many $\delta K{\textit{ir}}$ (7 of 10) also had contributions in the next
three groups, C2 through C4. Most \( \text{g}^\text{p}K_{ir} \) (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 \( \text{g}^\text{p}K_{ir} \) were fit well with five components, C1 through C5, and one other had both components C4 and C5. A group C6 component was observed for two \( \text{g}^\text{p}K_{ir} \) 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 \( \text{g}^\text{p}K_{ir} \) 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 \( \text{g}^\text{p}K_{ir} \) with spontaneous activity had...
burst-duration histograms fit by two exponentials (Fig. 10A). Prolongation of the longer bursts in the low-

P o mode compared with the medium-P o mode was consistent 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 o activity (Fig. 10A) suggests that a low proportion of events (<5%) occurred in the short open state, O s (Fig. 9A).

Excision led to increased P o of scK ir 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 C 1, an increase in C 2, and a lack of events in C 5 and C 6 (Fig. 10B). Excision appeared to have produced an increase in P o by recreating the kinetic conditions of the spontaneous medium-P o mode.

Reductions in P o by epinephrine and PGE 2 after forskolin stimulation (Fig. 9, G and H) occurred with a roughly equal and relatively large proportion of closures in C 2 and C 3. For another scK ir, PGE 2 reduced P o from a spontaneous medium-P o mode by decreasing the proportion of closures in C 2 and increasing the proportion in C 3 (data not shown) such that the final distribution was similar to that during forskolin/PGE 2 stimulation (Figs. 9H and 10B). Epinephrine and PGE 2 appeared to decrease P o largely through a shift of closed events into C 2 and C 3 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 cytoplasmic face of the channel. Notably, dependence on Ca 2+ and pH can be used to aid in distinguishing among intermediate-conductance Ca 2+ -activated K + channels (IK1; KCNN4), inward rectifier K + channels (Kir; KCNJ), and so-called background K + channels (TWIK; KCNK1). Activity of scK ir was similar with bath pH of 7.2 and 6.6 (Fig. 11A), whereas increasing bath pH to 8.1 reduced scK ir activity modestly (n = 3). Because renal epithelial K + channel (ROMK, Kir1.1; KCNJ1) activity decreases with acidification (9, 36), another channel type apparently was responsible for this scK ir observed in crypts. Reduction in bath solution free Ca 2+ (Fig. 11B) did not lead to lower activity for scK ir (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. Together with the K + concentration gradient developed by operation of the Na + -K + pump, basolateral membrane K + conductance serves to generate a cell-negative basolateral membrane electrical potential difference (V b). Apical membrane Cl − channels allow Cl − exit into the lumen, driven by the electrochemical gradient. 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, depends 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 + channels in both apical and basolateral membranes contribute to maintaining a cell negative V a that promotes Cl − secretion. In addition, K + conductance of apical membrane 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. Relative rates of Cl − and K + secretion vary among mammalian species (15), but in guinea pig distal colon these electrogentic flows are roughly equal when stimulated by high concentrations (>100 nM) of PGE 2 (17, 41). Activation by epinephrine (15, 41) or low concentrations (<100 nM) of PGE 2 (17, 41) produces electrogenic K + secretion without accompanying Cl − secretion. Cl − entering via basolateral membrane Na + -K + -2Cl − cotransporters during this type of sustained electrogenic K + secretion apparently exits across the basolateral membrane through Cl − channels (15, 30, 41). Control of basolateral K + channels during primary electrogenic K + secretion, thus, also would contribute to maintaining 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 portions 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 V m = 0 mV is 30, 16, and 28 pS for Kir1.1 (9), IK1 (23, 25), and TWIK (29), respectively. Specific sensitivities to pH, Ca 2+ , and ATP also contribute to a functional identification of these channel types (10). In particular, the ROMK channel (Kir1.1; KCNJ1) is inhibited by acidification 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$^{+}$ is a key feature of IK1 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$^{2+}$/H$^{+}$, 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 $g$ of 20–40 pS, also have been observed in basolateral membranes of colonic epithelial cells (4, 6, 42, 47), similar in size to those observed in guinea pig crypts. Large-conductance K$^+$ channels in crypts have $g$ ranging from ~100 pS to >200 pS, and some are

![Fig. 9. Secretagogue dependence of kinetics for $\kappa_\text{Kir}$. Histograms of open (A–D) and closed durations (E–H) are shown in which log binning was used (see METHODS) for a single $\kappa_\text{Kir}$ during cumulative addition of several secretagogues while cell-attached (see Fig. 7B). $V_{\text{hold}}$ was ~70 mV. Each current record was ~50 s in length with a various number of events in each condition: basal (740), forskolin (5,479), epinephrine (8,252), and PGE$_2$ (8,380). Open-duration histograms for basal and forskolin conditions had a peak fit well by a single exponential; open-duration histograms for epinephrine and PGE$_2$ conditions had a peak fit best by a mixture of 2 exponentials. Relative frequency was obtained by normalizing to the number of open events, estimated from the fitted exponentials. Individual fit components are shown as dotted lines. Fitted open time constants and proportions of events (in parentheses) were basal, 9.4 ms; forskolin, 7.7 ms; epinephrine, 1.1 ms (0.38) and 2.4 ms (0.62); and PGE$_2$, 0.7 ms (0.40) and 1.5 ms (0.60). Closed-duration histograms exhibited multiple peaks requiring a mixture of exponentials for an adequate fit; relative frequency was obtained by normalizing to the number of open events for that condition. Fitted closed time constants and proportions of events (in parentheses) were basal, 0.3 ms (0.94), 8.6 ms (0.03), 187 ms (0.02), and 14.5 ms (0.01); forskolin, 0.3 ms (0.87), 3.7 ms (0.06), 10.8 ms (0.06), and 101 ms (0.01); epinephrine, 0.3 ms (0.45), 2.3 ms (0.30), 6.6 ms (0.24), and 59.3 ms (0.01); and PGE$_2$, 0.3 ms (0.54), 3.4 ms (0.30), 8.3 ms (0.14), and 50.4 ms (0.01). Kinetic parameters from currents at $V_{\text{hold}}$ of ~80 mV were similar.
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; KCNA) 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 \(\sim 20\) 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 (<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\(^{+}\) (KCNE) (58). Identity of this crypt K\(^{+}\) channel as KvLQT1/minK\(^{+}\) (KCNQ1/KCNN4) 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, \(\text{gpKir}\) (Fig. 1B). A larger conductance K\(^{+}\) channel was observed rarely (Fig. 1D) that may correspond to the BK channel (slo; KCNA) (10). Identity of the \(\text{gpKir}\) observed in basolateral membranes is uncertain. With physiological external K\(^{+}\) and Na\(^{+}\) concentrations (Figs. 1A and 2), \(\text{gpKir}\) apparently had a linear current-voltage relation. A less commonly observed inwardly rectified K\(^{+}\) channel (Fig. 1C), which may be another conductance state of \(\text{gpKir}\), had steeper rectification than \(\text{gpKir}\) at negative \(V_m\) (Fig. 3E). Insensitivity to changes in Ca\(^{2+}\) activity on the cytoplasmic side of \(\text{gpKir}\) (Fig. 1B) suggests that IK1 (KCNN4) (10) is not a candidate protein unless other associating components could modify Ca\(^{2+}\) dependence. Insensitivity to acid pH (Fig. 1A) suggests that ROMK (Kir1.1; KCNJ1) (9, 36) also is not the protein forming \(\text{gpKir}\). The lack of these specific controlling factors for \(\text{gpKir}\) 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 \(\text{gpKir}\) (Figs. 3B). Thus \(\text{gpKir}\) does not conform easily to any of these known inwardly rectified K\(^{+}\) channel types.
Conduction (Fig. 3E) and activation (Fig. 11) properties for \( \sigma \) suggest that guinea pig colonic crypts exhibit a \( K^{+} \) channel distinct from those already characterized in basolateral membranes for this type of epithelial cell (55). However, several commonalities can be found between \( \sigma \) and previously observed intermediate-conductance \( K^{+} \) channels. ROMK has a subconductance state of \( 13 \) pS corresponding to a particular phosphorylated state (34). Coexpression of the \( Cl^{-} \) channel cystic fibrosis transmembrane conductance...
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 $P_o$ independent of $V_m$ (50), similar to that for $\text{Ca}^{2+}$-Kir (Fig. 4A). Other Ca²⁺-dependent K⁺ channels of intermediate conductance exhibit $P_o$ that increases (5) or decreases (56) at more positive $V_m$. ROMK has high $P_o$ (~0.9) that is relatively independent of $V_m$ (40). High relative permeability to Rb⁺ (Fig. 3) appears to distinguish $\text{Ca}^{2+}$-Kir from both ROMK (8) and IK1 (49). Although none of the currently established K⁺ channels (10) has identical characteristics to $\text{Ca}^{2+}$-Kir, a distinct assembly of channel and regulatory subunits (27) presumably confers the properties observed for $\text{Ca}^{2+}$-Kir.

**Regulation of Cl⁻ and K⁺ secretion.** Control of $\text{Ca}^{2+}$-Kir in the basolateral membrane participates in production of Cl⁻ and K⁺ secretion by contributing to maintenance of $V_m$ that drives Cl⁻ exit into the lumen while also adjusting the proportion of K⁺ exit into the lumen. Activation of $\text{Ca}^{2+}$-Kir 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 $\text{Ca}^{2+}$-Kir ($N_{Kr}$) and $P_o$ increased (Fig. 8) such that basolateral membrane K⁺ conductance ($g^K$) would increase: $g^K = N_{Kr}P_o γ^K$. The K⁺ secretagogues PGE₂ and epinephrine led to decreased $g^K$ primarily by reducing $P_o$ (Fig. 8) and occasionally by deactivating $\text{Ca}^{2+}$-Kir ($N_{Kr}$). 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 $\text{Ca}^{2+}$-Kir can be distinguished on the basis of the kinetics producing $P_o$. Spontaneous activity exhibited two modes, with low and moderate $P_o$ (Figs. 4A and 10). The cAMP-dependent agent forskolin produced moderate $P_o$ with kinetics different from the spontaneous mode (Figs. 8A, 9, and 10). The K⁺ secretagogues PGE₂ and epinephrine reduced $P_o$ to an intermediate level with kinetics distinguishable from the other three modes (Figs. 8A, 9, and 10). These four kinetic modes of $\text{Ca}^{2+}$-Kir presumably are produced by distinct regulatory mechanisms characteristic of the components making up this channel complex.

A kinetic scheme for $\text{Ca}^{2+}$-Kir 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 C₅ and C₆ groups were associated only with the low-$P_o$ mode. The medium-$P_o$ mode also differed from the low-$P_o$ mode by having similar contributions from C₁ and C₂. Intermediate $P_o$ of the K⁺ secretory mode occurred with a relatively high contribution from C₃. Both the low-$P_o$ and forskolin modes had open durations dominated by a single state with a time constant approximately four times longer than $\text{O}_L$, of the medium-$P_o$ and K⁺ secretory modes.

A common feature of many K⁺ channels, including $\text{Ca}^{2+}$-Kir, 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 $\text{Ca}^{2+}$-Kir 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 $P_o$ by decreasing the number of long-duration closures without changing open durations (9, 36). Heteromeric Kir4.1-Kir5.1 (KCNJ10–KCNJ16) also increases $P_o$ 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 $P_o$ can be controlled through a balance between kinase and phosphatase activity (13, 28, 39, 50, 54, 57).

The tendency for excision to increase $P_o$ of $\text{Ca}^{2+}$-Kir (Fig. 4) suggests that a cytoplasmic inhibitory component was lost. One possibility could be that a membrane-resident phosphatase (2) dephosphorylated $\text{Ca}^{2+}$-Kir 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 $P_o$ of $\text{Ca}^{2+}$-Kir into the range of the spontaneous medium-$P_o$ mode (Fig. 4A). In part, $P_o$ increased because $\text{Ca}^{2+}$-Kir did not enter long-lived closed states. However, during forskolin treatment $\text{Ca}^{2+}$-Kir openings were predominately to the longer open state (Fig. 9B), whereas after excision openings occurring to both states and $O_L$ 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 $P_o$ such that relieving the negative control is sufficient for increased activity.

Cholinergic activation of $\text{Ca}^{2+}$-Kir 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, Ca²⁺ dependence may be indirect, conferred perhaps via a Ca²⁺-activated kinase such as PKC or calmodulin-dependent kinase. Reduction of $\bar{g}_{K_p}$, $P_o$ by the K⁺ secretagogues PGE₂ 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 ~14% decrease of intracellular CAMP levels (45). Although PGE₂ and epinephrine can act through receptors that increase cytoplasmic cAMP (15), this inhibitory control of $\bar{g}_{K_p}$ may occur through another second messenger pathway such that several separable mechanisms may be involved in controlling $\bar{g}_{K_p}$ 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 cholinerigic 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 $\bar{g}_{K_p}$ 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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