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Ca^{2+} and cAMP Activate K^+ Channels in the Basolateral Membrane of Crypt Cells Isolated from Rabbit Distal Colon

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Summary. Using patch-clamp techniques, we have studied Ca^{2+}-activated K^+ channels in the basolateral membrane of freshly isolated epithelial cells from rabbit distal colon. Epithelial cell clusters were obtained from distal colon by gentle mechanical disruption of isolated crypts. Gigaohm seals were obtained on the basolateral surface of the cell clusters. At the resting potential (approximately −45 mV), with NaCl Ringer's bathing the cell, the predominant channels had a conductance of 131 ± 25 pS. Channel activity depended on voltage as depolarization of the membrane increased the open probability. In excised inside-out patches, channels were found to be selective for K^+ over Na^+. Channel activity correlated directly with bath Ca^{2+} concentration in the excised patches. Channel currents were blocked by 5 mM TEA^+ and 1 mM Ba^{2+}. In cell-attached patches, after addition of the Ca^{2+} ionophore A23187, which increases intracellular Ca^{2+}, open probability was markedly increased. Channel activity was also regulated by cAMP as addition of 1 mM dibutyryl-cAMP in the bath solution in cell-attached patches increased channel open probability over 20-fold. Channels that had been activated by cAMP were further activated by Ca^{2+}. We conclude that the basolateral membrane of epithelial cells from descending colon contains a class of potassium channels, which are regulated by intracellular Ca^{2+} and cAMP.

Key Words colon · ion transport · ion channel · cyclic nucleotides · calcium · potassium

Introduction

The colon, when stimulated, can secrete massive amounts of fluid. This observation belies the primary function of the colon, which is water and electrolyte absorption. The mechanism of colonic fluid secretion has been of intense interest over the past decade. It is generally accepted that fluid secretion occurs from the colonic crypts with a concomitant increase in chloride conductance and is induced by secretagogues which elevate intracellular concentrations of cAMP (Welsh et al., 1982). Recently, Frizzell and co-workers (1986) have shown that the human colon-cancer derived cell line T84, a model secretory epithelium, contains electrogenic, cAMP and Ca^{2+}-activated Cl^- channels in the apical membrane. Secretion thus may occur by active uptake of Cl^- across the epithelial cell basolateral membrane and passive Cl^- exit down the electrochemical gradient via apical Cl^- channels. Basolateral Cl^- entry is thought to occur by cotransport with Na^+ and K^+ (Heintze, Stewart & Frizzell, 1983; Dharmsathaphorn et al., 1985). For secretion to continue, excess cellular K^+, imported by the basolateral cotransporter and Na^+,K^+ ATPase must exit the cell, probably across the basolateral membrane.

At present, little is known about the channels in the basolateral membrane of the mammalian colon, perhaps owing to the inaccessibility of the epithelial cell basolateral membrane *in situ* (Wills, 1984). Data obtained from the study of transepithelial currents and intracellular recordings has been interpreted to be consistent with the presence of basolateral potassium conductive pathways in the rabbit colon (Wills, 1984; Wills et al., 1979*a*; Wills, Lewis & Eaton, 1979*b*), but the nature of these pathways remains to be elucidated. Prior to the present studies, single-channel recordings have not been performed on the basolateral membrane of mammalian colonic epithelial cells.

In this paper, we apply the patch-clamp technique to a novel preparation of cell clusters obtained from rabbit descending colon. By first isolating intact colonic crypts and then reducing the number of goblet cells by incubating the crypts, a preparation of viable crypt cell clusters is obtained. These isolated clusters are oriented so that the basolateral membranes face the bathing medium, facilitating approach by a patch pipette. The basolateral membrane of these glands contains a high density of Ca^{2+}-activated potassium channels, which

are also stimulated by cAMP. The physiologic significance of these channels will be discussed in the context of studies of intact epithelium and isolated channels.

Materials and Methods

Cell Preparations

Isolated cells were obtained from a preparation of pure epithelial glands prepared from rabbit descending colon in a modification of a previously described method (Kaunitz, 1988). Stripped, minced mucosa was digested as previously described with the exception that digestions were carried out in Hank's solution containing 1.0% rather than 0.1% bovine serum albumin. Isolated glands were incubated in a 5% CO_2 37°C incubator with gentle stirring in Ham's F12/Dulbecco's minimal essential medium 1 : 1 containing 1 mM glutamine and 5% newborn calf serum for 3 hr. It was found that freshly isolated glands resisted dissociation by rather violent means (e.g., vortexing), whereas incubated glands were easily dissociated into grape-like cell clusters with gentle agitation. Dissociated glands were stored on ice until use and were suitable for patch-clamp experiments up to 10 hr after preparation.

For patch-clamp studies, an aliquot of cell clusters was pipetted into a 0.5 ml glass bottom chamber on the stage of a Nikon inverted microscope with Hoffman-modulated optics. After the cells were allowed to settle on the bottom, the chamber was perfused with several volumes of Ringer solution using a peristaltic pump. Clusters that adhered to the bottom of the chamber were found to yield the highest seal resistances.

Solutions

In all experiments, the pipette was filled with a KCl solution that contained (in mM): 140 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 ethyleneglycol-*bis*-(β-aminoethyl ether)-N,N,N',N',-tetraacetic acid (EGTA) and 10 N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (HEPES)/KOH (pH 7.3). The free-Ca^{2+} concentration was calculated to be 5 nM. The solution bathing the cells was (in mM): 140 NaCl, 1 $MgCl_2$, 1 $CaCl_2$ and 10 HEPES/NaOH (pH 7.3). In some experiments, bath Ca^{2+} was lowered to 5 and 500 nM, prepared by using Ca^{2+}-EGTA buffers at concentrations determined previously (Ueda, Loo & Sachs, 1987). For a free Ca^{2+} = 5 nM, $CaCl_2$ was 1.0 mM and EGTA was 10.0 mM; for a free Ca^{2+} = 500 nM, $CaCl_2$ was 1.16 mM and EGTA was 1.34 mM. The pipette solution was filtered with 0.22 μm nitrocellulose filters prior to use.

A23187 (calcium-magnesium salt, Sigma) was dissolved in DMSO at a stock concentration of 2 mM, and stored at 0°C, and diluted by NaCl Ringer's into a final concentration of 2 μM before experimental use. Solutions containing 1 mM dibutyryl cyclic AMP (N^6,2'-O-dibutyryladenosine 3' : 5'-cyclic monophosphate monosodium salt, Sigma) were prepared prior to each experiment by dissolving the salt directly into the Ringer's solution. All experiments were done at room temperature (20–23°C).

Electrophysiological Methods

Pipettes with tip resistances of 5–10 MΩ were pulled from Corning 7052 glass and fire-polished. The patch-clamp methods used were as previously described (Ueda et al., 1987; Brown, Loo & Wright, 1988). Single-channel activity was studied by holding the isolated membrane patches (cell-attached and inside-out) at constant DC potentials. All potentials are expressed with respect to the bath solution. Making the potential of the pipette (V_p) positive in cell-attached and excised inside-out patches corresponded to hyperpolarizations of the cell membrane from the resting potential. For *I-V* (current-voltage) plots in cell-attached patches, the abscissa is represented by ($-V_p$) and indicates depolarizations from the resting membrane potential.

Data Analysis

Single-channel data were stored on an analog tape recorder and later replayed for analysis using the computer program "*p*Clamp" (Axon Instruments, Burlingame, CA). Data were digitized and analyzed using an IBM personal computer interfaced to the Tecmar Labmaster data acquisition system (Cleveland, OH). Prior to digitization, signals were filtered with an eight-pole, low-pass Bessel filter (Model LP902, Frequency Devices, Haverhill, MA) with corner frequency set to 1 or 3 kHz. Steady-state portions of the current signal were then digitized at the rate of 200 μsec (5 kHz) and stored in the computer.

A modified "*p*Clamp" program was used to analyze single-channel events. The modification allowed us to set the baseline and threshold levels (Brown et al., 1988). A section of the digitized record was displayed on a graphics monitor, and the threshold level set at 50% of a channel opening. An event was considered acceptable if the current remained above this value for >400 μsec (at least 3 digitized points). The program calculated the open time, the closed time, and the single-channel currents. In general, 30 sec of recorded data were analyzed by the computer for each potential. For open probabilities $\leq 10^{-3}$, at least 2 min of data were analyzed. Usually, 100 events or more were analyzed to measure single-channel currents as plotted in the current-voltage relations. In situations in which the signal/noise was low (i.e., small current steps), analysis was done by eye directly on the oscilloscope screen using a Nicolet digital oscilloscope. Multiple current steps were resolved using the "*p*Clamp" computer program and with the digital oscilloscope.

The open probability (P_o) of a single channel was defined as the fraction of the total time the channel was open and was estimated in multichannel patches using the following equation (Reuter et al., 1982; Gallacher & Morris, 1986; Maruyama, Matsunaga & Hoshi, 1986; Brown et al., 1988):

$$P_o = (T_1 + T_2 + \ldots + T_N)/NT_{tot} \quad (1)$$

where N = number of channels in the patch, T_{tot} was the total time analyzed, and T_1 is the time that one or more channels are open, T_2 is the time two or more channels are open and so on. N was found empirically by counting the maximum number of current steps observed under conditions (depolarizing potentials) at which the channels show their greatest activity. T_1, T_2, . . . T_N were estimated by setting the baseline and current thresholds at progressively higher levels from 1 to N. Any P_o values calculated to be lower than 10^{-3} are expressed as $P_o < 10^{-3}$. All statistics were expressed as mean ± SEM.

Results

Characterization of Isolated Crypts

Figure 1*A* shows a preparation of intact colonic glands, consisting of a tuft of surface cells attached

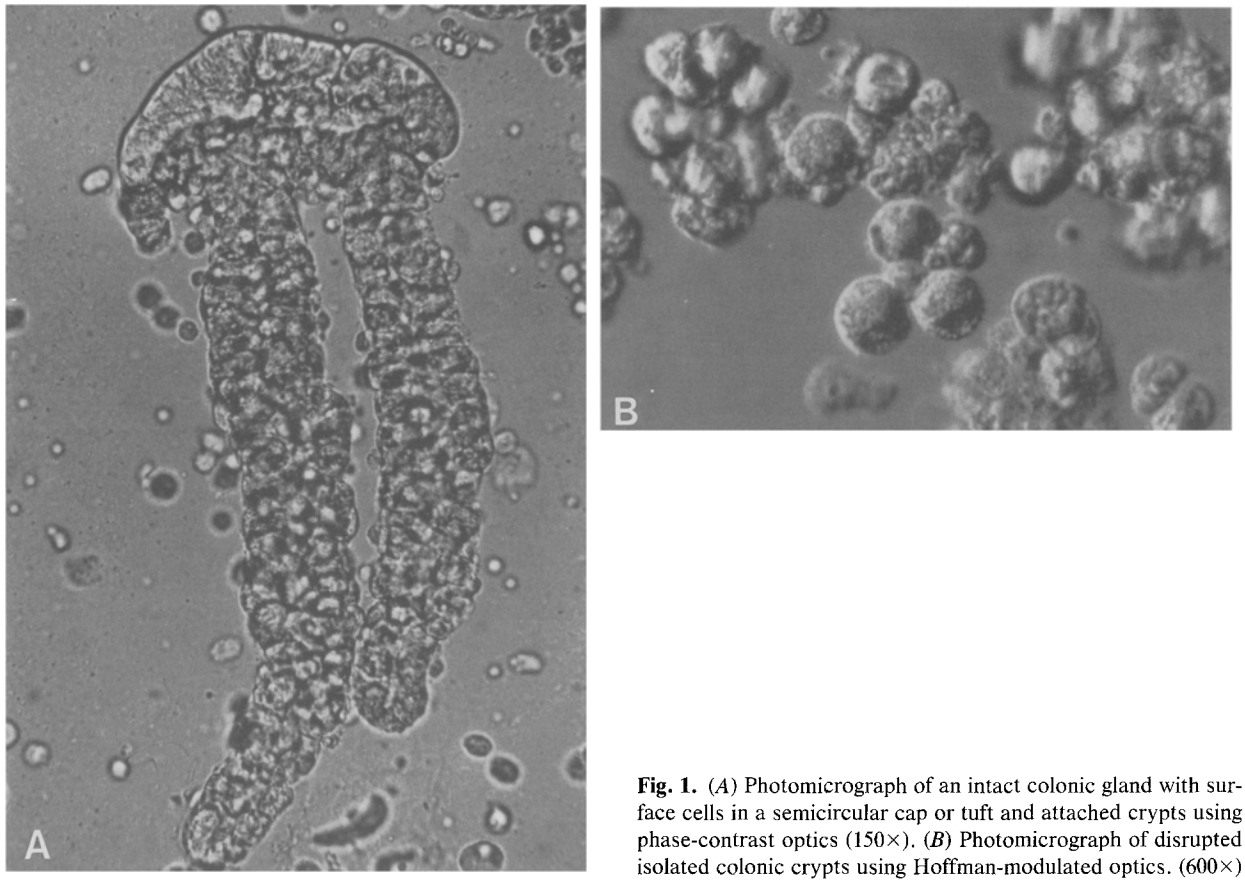

Fig. 1. (*A*) Photomicrograph of an intact colonic gland with surface cells in a semicircular cap or tuft and attached crypts using phase-contrast optics (150×). (*B*) Photomicrograph of disrupted isolated colonic crypts using Hoffman-modulated optics. (600×)

to intact crypts. Incubation of glands for 3 hr at 37°C dispersed the surface cells, leaving intact crypt fragments (Fig. 1*B*). These fragments were suitable for patch-clamp studies for the following reasons: first, the morphology of the crypt cells was easily recognized, with the basolateral surface exposed to the medium, and apical membrane facing a central lumen. Electron microscopy (courtesy Deborah Anderson, CURE) confirmed the intact morphological arrangement of the crypt cells. Second, incubation reduced the number of goblet cells to 10%. Since goblet cells and columnar cells are the only major epithelial cell types in colon, incubation increased the likelihood that ion-transporting cells rather than mucous cells were patched. Third, crypts were easily disrupted. Prior to incubation, intact glands were difficult to disrupt. It was found that whole crypts were too large to permit an effective approach by the patch pipette, and yielded unstable patches, whereas smaller crypt fragments were much more tractable. Seals (typically 10–30 GΩ) could be formed on the exposed basolateral surface in crypt fragments. In cell-attached patches, most of the patches contained a class of channels with large unitary current steps, which were activated by membrane depolarization. In cell-attached patches, most of the patches contained a class of channels with large unitary current steps, which were activated by membrane depolarization. These large channels are the subject of the present study.

Channel Conductance

Channels were first studied in the cell-attached configuration with the pipette solution containing 140 mM KCl and 5 nM Ca^{2+}, and the bath solution containing 140 mM NaCl and 1 mM Ca^{2+}. In the cell-attached configuration, channel openings appeared as discrete inward current steps (Fig. 2*A*). At the resting potential ($V_p = 0$) P_o was $2.54 \pm 0.90 \times 10^{-2}$ ($n = 7$), with a range of 0.11×10^{-2} to 6.9×10^{-2}. With depolarization of the patch (more negative pipette potential V_p), the size of the current steps decreased and the frequency of channel openings increased. For instance, at $V_p = -20$ mV, several steps became prominent (Fig. 2*A*). On the average, most patches contained at least three channels. When the pipette potential was close to −50 mV, the current steps were small and the multiple events were so frequent that it was sometimes difficult to distinguish the individual current steps.

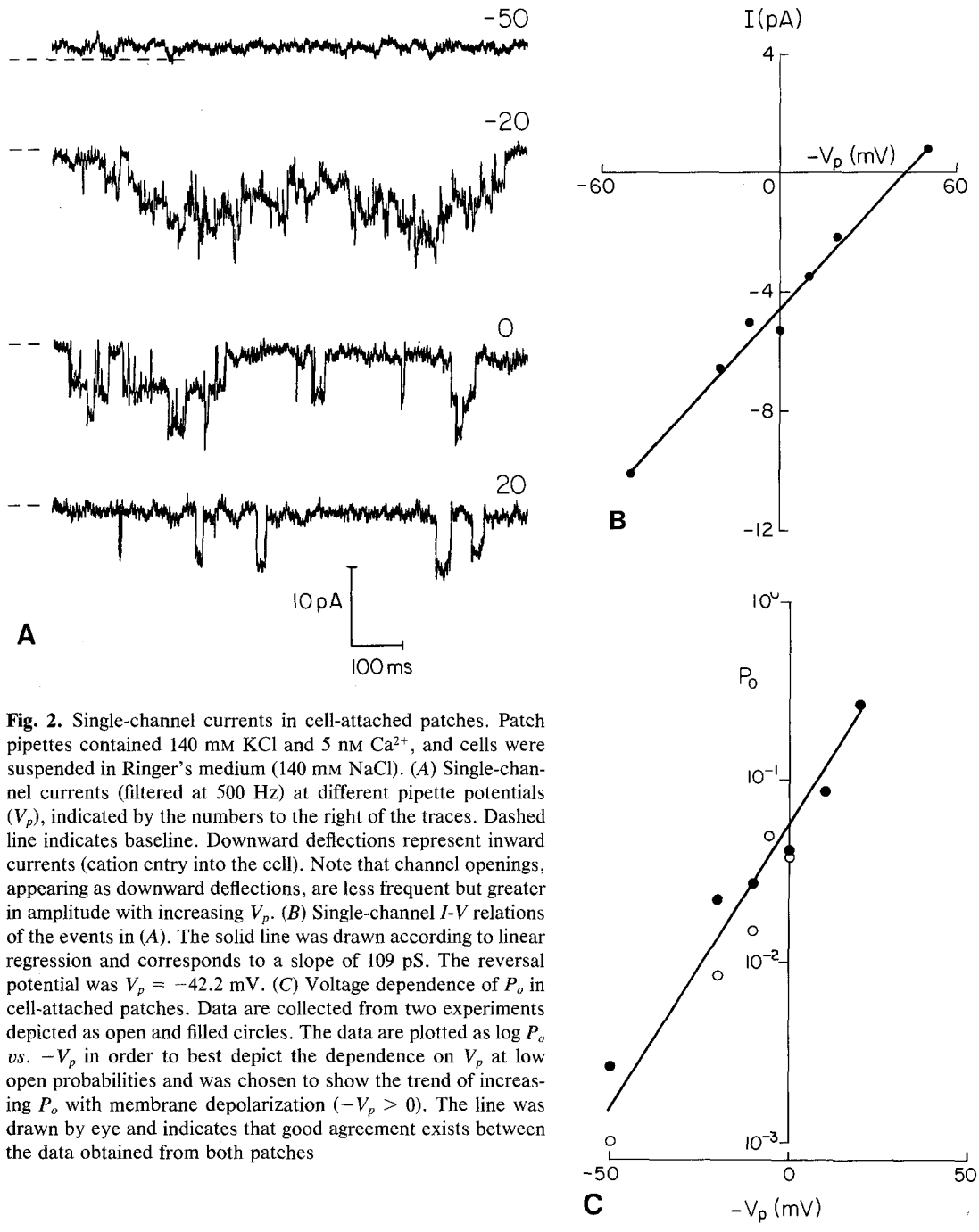

Fig. 2. Single-channel currents in cell-attached patches. Patch pipettes contained 140 mM KCl and 5 nM Ca^{2+}, and cells were suspended in Ringer's medium (140 mM NaCl). (*A*) Single-channel currents (filtered at 500 Hz) at different pipette potentials (V_p), indicated by the numbers to the right of the traces. Dashed line indicates baseline. Downward deflections represent inward currents (cation entry into the cell). Note that channel openings, appearing as downward deflections, are less frequent but greater in amplitude with increasing V_p. (*B*) Single-channel *I-V* relations of the events in (*A*). The solid line was drawn according to linear regression and corresponds to a slope of 109 pS. The reversal potential was $V_p = -42.2$ mV. (*C*) Voltage dependence of P_o in cell-attached patches. Data are collected from two experiments depicted as open and filled circles. The data are plotted as log P_o *vs.* $-V_p$ in order to best depict the dependence on V_p at low open probabilities and was chosen to show the trend of increasing P_o with membrane depolarization ($-V_p > 0$). The line was drawn by eye and indicates that good agreement exists between the data obtained from both patches

The current-voltage (*I-V*) relationship for the cell-attached patch depicted in Fig. 2*A* is shown in Fig. 2*B*. *I-V* relations were linear in the range of V_p's studied (from +50 to −50 mV). The line in Fig. 2*B* was drawn by linear regression analysis and yielded a conductance of 109 pS and a reversal potential of −42.2 mV. The means for the single-channel conductance and reversal potential from five experiments was 131 ± 25 pS (range 90–220 pS) and −46 ± 5 mV, respectively. This reversal potential would be close to the resting potential of the cells if the pipette and intracellular K^+ concentrations are the same. Experiments with excised patches were used to characterize the ion selectivity of these channels (*see below*).

Channel activity increased as membrane potential was made more positive. Figure 2*C* depicts log P_o plotted as a function of V_p from two experiments

in the cell-attached configuration. When P_o is plotted against V_p using linear coordinates, the relationship is sigmoidal. Using the relation $(V_p) = 1/[1 + k(V_p - V_{0.5})]$ described by Wong, Lecar and Adler (1982), we found that $V_{0.5}$ (voltage where $P_o = 0.5$) and k (slope parameter) were -39 mV and 0.069 $(mV)^{-1}$, respectively, for the channels depicted in Fig. 2*C*. The means for $V_{0.5}$ and k from eight experiments were -73 ± 26 mV and 0.072 ± 0.008 $(mV)^{-1}$, respectively.

Selectivity for K^+ over Na^+

The K^+ selectivity of the channels was studied by determining the reversal potential at different K^+ concentration gradients across the membrane in excised inside-out patches (Fig. 3). The solid circles are from an experiment on a 161 pS channel where the pipette and bath solutions contained symmetrical KCl (140 mM). With this condition, the single-channel *I-V* relation was linear (solid line) with the *y* intercept equal to 0 mV. The open circles represent the results of another experiment on a 109 pS channel under near bi-ionic conditions when the bath solution contained 140 mM NaCl and 2 mM KCl and the pipette solution contained 140 mM KCl. With this condition, the *I-V* relation was curvilinear (dashed line), and no current was observed to flow into the pipette, even at large negative pipette potentials. This rectification is consistent with a channel highly selective for potassium over sodium ($P_K/P_{Na} > 20$).

In many excised patches and most notably in experiments where the bath solution was changed several times, K^+ channels were unstable. With continual perfusion of the bath solution, an irreversible decrease in single-channel conductance occurred, suggesting that the K^+ channel may be composed of several subconductance states.

Ca^{2+} Sensitivity

The results of an experiment where the channels were studied in the cell-attached configuration with the addition of the Ca^{2+} ionophore A23187 to the bath solution is illustrated in Fig. 4. The left panel shows the activity of the channels as first studied when the cells were bathed in Ringer's solution (Ca^{2+} = 1 mM). Two representative tracings recorded at V_p's of 0 and 20 mV are shown. For a V_p of 0 mV, P_o was 1.1×10^{-3} increasing 400-fold to 0.44 after addition of A23187. When V_p was -20 mV, $P_o < 1.0 \times 10^{-3}$, increasing > 100-fold to 0.12 after application of A23187.

The effect of Ca^{2+} was also studied in excised

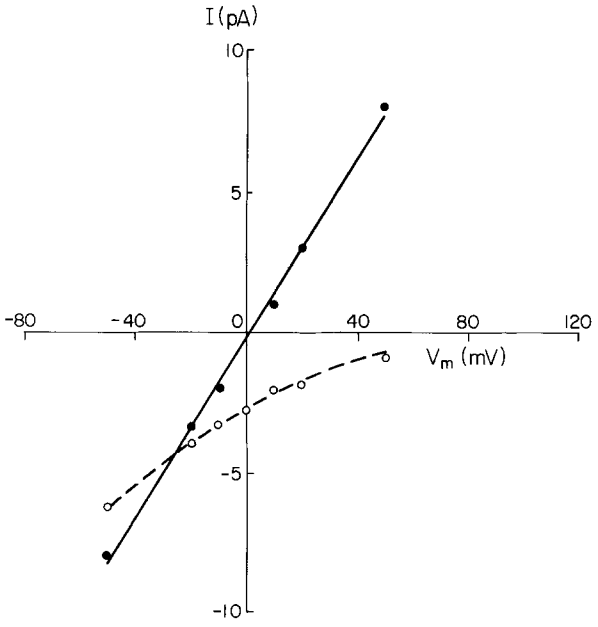

Fig. 3. Ion selectivity of channels in excised inside-out patches. Solid circles are the *I-V* relations when the pipette and bath solutions contained 140 mM KCl. Pipette Ca^{2+} was 5 nM whereas bath contained 500 nM Ca^{2+}. The straight line was drawn by linear regression and has a slope of 161 pS. Open circles are the *I-V* relations obtained when the bath solution contained 140 mM NaCl and 2 mM KCl and the pipette contained 140 mM KCl. The data were obtained with the same 109-pS channel depicted in Fig. 2*A* and *B*. The dashed curve was drawn by eye. The calculated reversal potential for K^+ was -107 mV. In similar experiments done on other patches, no reversal was observed at V_p = 70 mV

inside-out patches where the bath calcium concentration was altered. This method allowed us to separate the direct effects of Ca^{2+} from possible secondary effects of Ca^{2+} ionophore such as pH changes, and it was also possible to remove the influence of cytosolic mediators. In the top tracing of Fig. 5, an excised inside-out patch was exposed to solutions containing 140 mM KCl in bath and pipette and containing 500 nM calcium in the bath. The activity of five channels can be seen, yielding a P_o of 0.6. In the lower panel, bath Ca^{2+} was reduced to 5 nM. Channel activity is drastically reduced, yielding a $P_o < 10^{-3}$, a >600-fold decrease.

From these experiments, we conclude that the channels in the membrane patch were activated by increasing the Ca^{2+} concentration bathing the cytosolic face of the patch.

TEA^+ and Ba^{2+} Block

Ca^{2+}-activated K^+ channels are typically blocked by TEA^+ and Ba^{2+} (Latorre & Miller, 1983). We

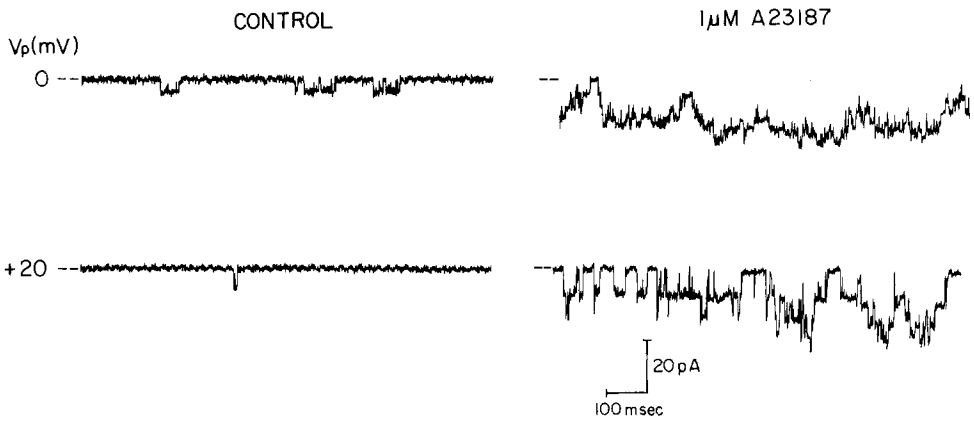

Fig. 4. Activation of K^+ channels in a cell-attached patch by intracellular Ca^{2+}. The pipette solution contained 140 mM KCl and 5 nM Ca^{2+} and the bath solution was 140 mM NaCl Ringer. The traces to the left indicate control conditions, shown at two pipette potentials, 0 and 20 mV. The dashed line indicates the baseline. The bath solution was replaced by the same NaCl Ringer solution containing 1 μM A23187, and the channel activity for the same two pipette potentials are shown at the right. There is a large increase in channel activity after addition of the ionophore. The current records were filtered at 500 Hz

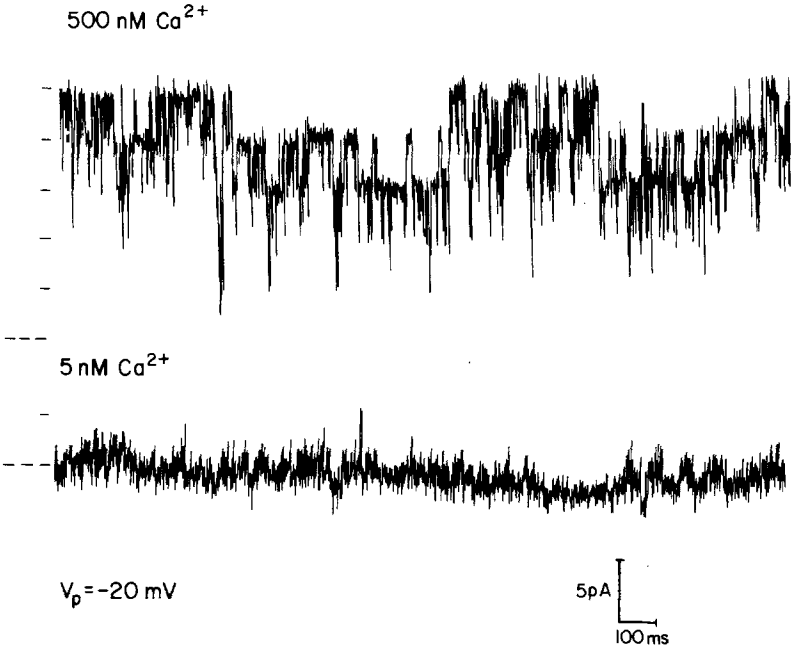

Fig. 5. Ca^{2+} sensitivity of K^+ channels in excised inside-out patches. The patch was held at $V_p = -20$ mV. Pipette solution contained 140 mM KCl and 5 nM Ca^{2+}. Single-channel conductance was 220 pS. Channel activity was first studied with bath containing 140 mM KCl and 500 nM Ca^{2+} (upper trace). The bath solution was then replaced by a solution containing 140 mM KCl and 5 nM Ca^{2+} (lower trace). One channel opening is present in the lower trace, and background noise is increased from the low Ca^{2+} concentration used. The dashed line indicates the baseline (all channels closed), and smaller lines represent the amplitude of each current step

investigated the sensitivity of single K^+ channels in excised patches to TEA^+ and Ba^{2+}. When added to the inside of the patch, we found that 5 mM TEA^+ completely abolished the activity of the Ca^{2+}-activated K^+ channel in colon crypt cells. Channel activity was blocked by 1 mM Ba^{2+} when added to either the outside or inside of the membrane patch (*data not shown*).

Sensitivity to cAMP

Sensitivity of the K^+ channels to cAMP was studied in cell-attached patches by the addition of 1 mM dbcAMP to the bath solution. Figure 6 illustrates the results of such an experiment on a 197-pS K^+ channel. The pipette solution contained 140 mM KCl with 5 nM Ca^{2+} and bath solution was 140 mM NaCl Ringer (1 mM Ca^{2+}). The numbers to the right of the current records indicate the time elapsed after the bath solution was replaced by the same Ringer solution containing 1 mM dbcAMP. P_o increased after an initial 9 min lag period from an initial value of 0.0084 to a steady state of 0.15 after 30 min. Every patch that contained K^+ channels was sensitive to cAMP ($n = 4$). Conversely, cAMP failed to elicit K^+ channel activity in "silent"

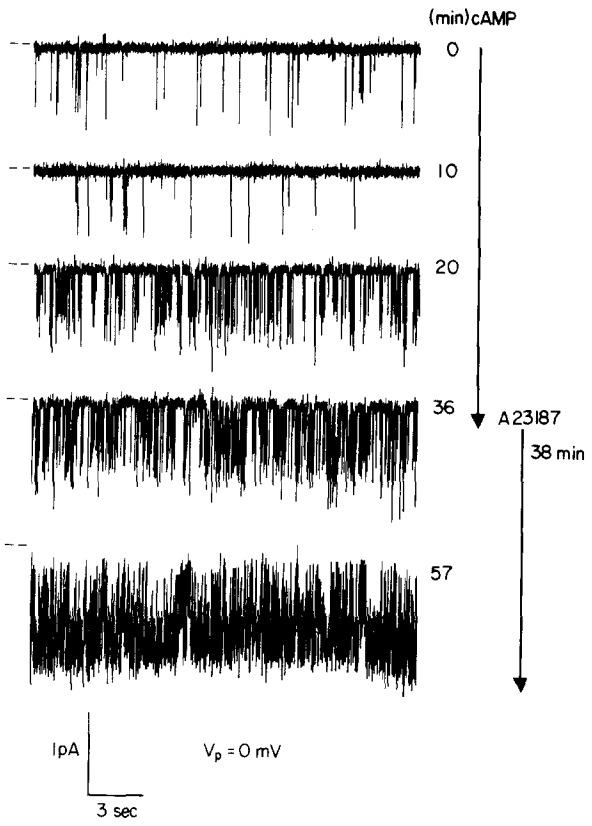

Fig. 6. Activation of K^+ channels by cAMP. The experiment was performed in the cell-attached configuration with the pipette containing 140 mM KCl and 5 nM Ca^{2+}. The bath solution contained 140 mM NaCl Ringer. The patch was held at the resting potential (V_p = 0 mV). The bath was then perfused with the same Ringer solution containing 1 mM dbcAMP. The numbers to the right of the current traces indicate the elapsed time after exposure to dbcAMP. After 38-min exposure, the bath solution was replaced by a Ringer solution containing 1 μM A23187. Current records were filtered at 500 Hz. Baseline is indicated by the dashed line to the left of the current traces. Similar results were obtained in four experiments. The compressed time scale was used to illustrate channel activity over a longer period. Though channel openings appear as 'spikes', they are similar to those shown in Figs. 2 and 4 when viewed with similar time scales

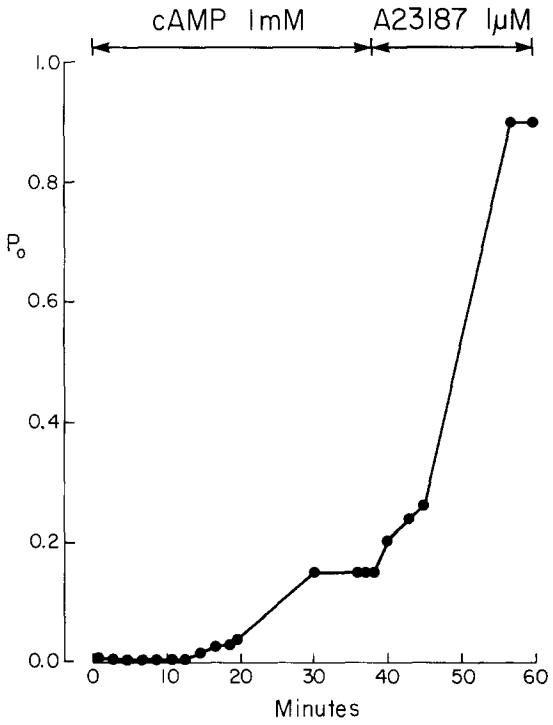

Fig. 7. Time course of changes in open probability P_o after exposure to 1 mM dbcAMP and 1 μM A23178. Patches were held at the resting potential (V_p = 0 mV). Data are from the same experiment as Fig. 6. P_o was evaluated by analyzing 30.72 sec of consecutive records. Similar results were obtained in four experiments

patches, in which no K^+ channels were detected, even after 30 min exposure (n = 4). cAMP thus increased "maxi" K^+ channel activity in the basolateral membrane of colonic crypt cell clusters to a steady state after 30 min.

Combined Sensitivity

To determine whether the channels, which had been activated by cAMP, were still sensitive to Ca^{2+}, the cAMP-containing Ringer's solution was replaced by one containing 1 μM A23187 after 38 min. of exposure, when channel activity had stabilized (Fig. 6, bottom trace; Fig. 7). A23187 produced a sudden increase in P_o, which reached a value of 0.9 after 25 min exposure to the ionophore. Voltage activation of the K^+ channels was sigmoidal after application of cAMP, with P_o increased an average of 20-fold at each V_p. After A23187, channel openings were so frequent that we were able to observe channel closures only at the resting potential and hyperpolarizing voltages. For example, for V_p = 0, P_o was 0.0084 in the control, 0.15 after cAMP (35-min exposure) and 0.9 after A23187 (25-min exposure).

I-V relations for the channel prior to stimulation (solid circles) and after stimulation by 1 mM dbcAMP (open circles) and 1 μM A23187 (solid squares) are shown in Fig. 8. A regression line calculated from control values is shown. Channel conductance was identical after stimulation with either agent. We thus conclude that Ca^{2+} and cAMP activate the same K^+ channel, though Ca^{2+} is more potent than cAMP.

Discussion

It has been hypothesized, on the basis of isotopic flux and electrophysiological measurements (Wills

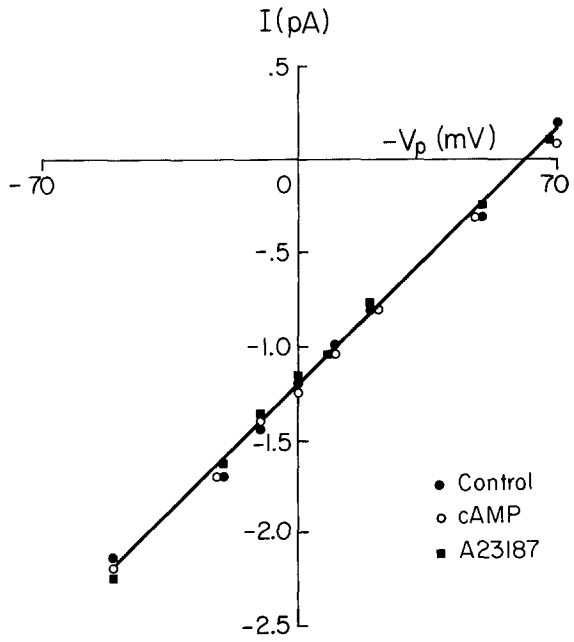

Fig. 8. Effect of cAMP and Ca^{2+} on channel conductance. Data are from the experiment of Fig. 6 and shows the *I-V* relations in control conditions (solid circles), after stimulation by 1 mM dbcAMP (open circles) and 1 μM A23187 (solid rectangles). The line was obtained by linear regression of control data. The slope is 197 pS and the *x* intercept is −62 mV. Linear regression after dbcAMP stimulation yielded a slope of 193 pS and an *x* intercept of −64 mV, and after A23187 stimulation was 196 pS and −62 mV. There was no change in conductance after stimulation by dbcAMP and A23187

et al., 1979*a,b;* Wills, 1984) that the colonic epithelial crypt cell basolateral membrane contains potassium conductive pathways. For instance, Wills et al. (1979*a,b*) used the permeabilizing antibiotic nystatin and elevated medium potassium to decrease apical membrane resistance so that the properties of the basolateral membrane could be studied. These studies, however, have shortcomings in that all measurements were either made across the whole epithelium or impaled surface cells, making it difficult to separate the contribution of the crypt cell basolateral conductance to the electrophysiology of the colonic epithelium. Our preparation of isolated colonic crypts, which are oriented with basolateral membrane outward, is therefore of interest in that it enables direct access to the crypt cell basolateral membrane. Individual channel-recording methods have allowed us to show that the putative potassium conductances, inferred from indirect studies, are large-conductance potassium channels.

Colonic crypt fragments were viable by many criteria. With the patch-clamp technique, we were able to confirm our previous findings that these cells maintained a high resting potential (E_m). E_m and intracellular potassium concentration in isolated colon crypt cells were found to be −52 ± 2 mV and 128 ± 8 mM, respectively, when measured with fluorescent dyes (Kaunitz, 1988). Since calculated intracellular potassium and pipette potassium concentrations (140 mM) were slightly asymmetric in the present experiments, we calculate that a small Nernst potential of +2.3 mV offsets the resting potential. We would thus predict a reversal potential of −52 + 2.3 mV = −49.7 mV. This corresponds well to the value of −46 ± 5 mV measured in the present experiments. We thus can conclude that the isolated colonic crypt preparation used consists of functional, viable cells.

Basolateral potassium channels in colonic glands fulfill the criteria of "maxi" K-channel as defined by Latorre and Miller (1983). Criteria include conductance 130–230 pS with 150 mM potassium bathing each membrane face, activation by calcium and depolarization, selectivity for potassium over sodium, and barium block. In the present study, channels in the colon crypt cell plasma membrane were, in addition to their Ca^{2+} sensitivity, activated by cAMP.

Agents that induce cAMP production or elevate intracellular calcium induce chloride secretion in rabbit distal colon. Frizzell (1977), in his studies of chloride secretion across rabbit distal colon, noted that application of cAMP or A23187 increased electrogenic chloride secretion. Welsh et al. (1982) found that prostaglandin E_2, a secretagogue which increases intracellular cAMP, promoted the appearance of fluid droplets in the crypt openings in oil covered rabbit colon. From these and subsequent studies of intact colon, it has been concluded that most secretagogues enhance Cl^- secretion from crypt cells.

A Ba^{2+}-blocked basolateral K^+ conductive pathway may play a role in colonic Cl^- secretion. For instance, addition of Ba^{2+} or increasing K^+ concentration in the serosal solution abolished electrogenic chloride secretion across intact rabbit colon (McCabe, Cooke & Sullivan, 1982; Plass, Gridl & Turnheim, 1986; Heintze et al., 1983), indicating that basolateral K^+ channels may be linked to chloride secretion. K^+ channels may promote chloride secretion by maintaining membrane potential and providing a "leak" pathway for excess potassium entering the cell via the Na^+,K^+-ATPase and sodium-chloride cotransport. It is thus logical to conclude that secretagogues that stimulate Cl^- secretion also activate basolateral potassium channels, in agreement with our findings.

Can "maxi" K^+ channels account for the basolateral K^+ conductance in colonic crypt cells?

There are no direct electrophysiological studies of the basolateral conductance of colon crypt cells. We have conducted preliminary whole-cell recording experiments in order to obtain an estimate of whole crypt-cell conductance. The whole-cell potassium conductance is barium-blocked, indicating that the "maxi" channels contribute to the whole-cell K^+ conductance. Our patch-clamp studies can be compared with the whole-tissue studies of rabbit distal colon. According to Sakmann and Neher (1983), the area of exposed membrane for a pipette with a resistance of 5–10 MΩ is 2–10 μm^2. Assuming an average of three channels/patch, the channel density was 0.3–1.5 μm^{-2}, which contrasts with the estimate of 12 μm^{-2} obtained by fluctuation analysis (Wills, 1984). Furthermore, Wills (1984) estimated a single-channel current of 0.01 pA under bi-ionic conditions at 0 mV, which differs from our measurement of 2.5 pA. The estimate of basolateral membrane P_K/P_{Na} in nystatin-treated colon obtained from whole tissue (Wills et al., 1979*a*) was 16, comparing well with our estimate of $P_K/P_{Na} \geq 20$. The agreement in selectivity data between our experiments and whole-tissue data support the notion that large-conductance, highly selective K^+ channels contribute to the macroscopic basolateral membrane conductance of the colonic epithelium.

Of particular interest are the effects of Ca^{2+} and cAMP on channel activity. Ca^{2+} increased activity is excised, inside-out patches and in intact cells. Activation by Ca^{2+} was rapid in either configuration. The activation of channel activity in excised patches suggests that at least part of the chloride-secretory apparatus is independent of cytosolic mediators such as protein kinase C, which has been implicated as being necessary for chloride secretion across rat colon (Donowitz, Cheng & Sharp, 1986).

The effect of cAMP on K^+ channels contrasted to that of Ca^{2+} in that activation was quite slow. A lag of 10 min was necessary before any effect could be seen and a steady increase in P_o was subsequently observed. Despite the large increase in P_o induced by cAMP, voltage increased P_o in a sigmoidal fashion. The conductance and reversal potential of the channels in the control state or after application of cAMP or calcium were identical. These results indicate that calcium and cAMP activate the same channels seen in resting conditions. These findings compare well with the whole-tissue studies of Frizzell (1977). For example, Frizzell (1977) found that the maximal effect of A23187 on I_{sc} occurred in less than 10 min, whereas the effect of cAMP on I_{sc} was gradual, with full effect occurring only after 20–30 min, with half-maximal effect occurring at about 5 min. The activation by cAMP in our system was slower than in intact tissue, perhaps reflecting the fact that our experiments were done at 22 rather than 37°C. The reason for the delayed effect of cAMP in the intact and isolated preparations is not clear, but may involve slow permeation of the cyclic nucleotide into the cell or rate-limiting intermediate steps. From our studies, Ca^{2+} and cAMP activate the same channel, though P_o was higher after Ca^{2+} than cAMP, in agreement with the work of Frizzell (1977), who found the increment in I_{sc} after A23187 was approximately 60% higher than after cAMP.

cAMP may activate channels either by increasing the calcium concentration of the cytosolic compartment bathing the channels or by phosphorylation of the channel protein or associated structures. Though good evidence exists that phosphorylation may regulate the activity of potassium channels in excitable tissues (Levitan, 1985), there has been little related experience with epithelial cells. Guggino et al. (1985) found large-conductance, voltage- and Ca^{2+}-activated potassium channels in cultured chick kidney cells stimulated by the adenylate cyclase activator forskolin or antidiuretic hormone. Patch-clamp techniques have been applied to isolated colon cells in a few previous studies. Richards and Dawson (1986) identified K^+ channels with conductances of 17 and 47 pS and blocked by Ba^{2+} in the plasma membrane of hyaluronidase-isolated turtle colon cells. These channels were not voltage-sensitive and their Ca^{2+} sensitivity was not determined, and thus contrast with those of the rabbit, perhaps reflecting underlying differences between the two species.

Electrogenic Cl^- secretion in secretory epithelia follows a similar paradigm in a variety of tissues. In a model proposed by Petersen (1986) for exocrine glands, Cl^- actively enters the cell across the basolateral membrane by cotransport with Na^+ and K^+, reaching a concentration above its equilibrium potential. The energy for active chloride transport is provided by the Na^+,K^+ ATPase, which produces an inward sodium gradient. Chloride exits the cell via apical Cl^- channels. Basolateral membrane potential and cell volume is maintained by K^+ efflux via Ca^{2+}-activated K^+ channels. Such a model may also apply to colonic Cl^- secretion. Studies of rabbit distal colon (Heintze et al., 1983) and cultured T84 human colon cancer cells (Dharmsathaphorn et al., 1985) have supported the presence of a basolateral Na-K-Cl_2 cotransport system. Patch-clamp studies have shown that Ca^{2+} and cAMP activate Cl^- channels in the apical membrane of T84 cells (Frizzell et al., 1986). Our finding of K^+ channels in the colon crypt cell basolateral membrane activated by the second messengers cAMP and Ca^{2+} further supports this model of Cl^- secretion.

In conclusion, we have demonstrated Ca^{2+}- and cAMP-activated voltage regulated K^+ channels in the plasma membrane of freshly isolated colonic crypt cells. These channels most likely reside in the basolateral membrane and may play a role in secretagogue-activated electrogenic Cl^- secretion.

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