Characterization of apical potassium channels induced in rat distal colon during potassium adaptation

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1. Chronic dietary K^+ loading stimulates an active K^+ secretory process in rat distal colon, which involves an increase in the macroscopic apical K^+ conductance of surface epithelial cells. In the present study, the abundance and characteristics of K^+ channels constituting this enhanced apical K^+ conductance were evaluated using patch clamp recording techniques.

2. In isolated non-polarized surface cells, K^+ channels were seen in 9 of 90 (10%) cell-attached patches in cells from control animals, and in 247 of 437 (57%) cell-attached patches in cells from K^+-loaded animals, with a significant ($P < 0.001$) shift in distribution density. Similarly, recordings from cell-attached patches of the apical membrane of surface cells surrounding the openings of distal colonic crypts revealed identical K^+ channels in 1 of 11 (9%) patches in control animals, and in 9 of 13 (69%) patches in K^+-loaded animals.

3. In isolated surface cells and surface cells *in situ*, K^+ channels had mean slope conductances of 209 ± 6 and 233 ± 14 pS, respectively, when inside-out patches were bathed symmetrically in K_2SO_4 solution. The channels were sensitive to 'cytosolic' Ca^{2+} concentration, were voltage sensitive at 'cytosolic' Ca^{2+} concentrations encountered in colonic epithelial cells, and were inhibited by 1 mM quinidine, 20 mM TEA or 5 mM Ba^{2+} ions.

4. The data show that dietary K^+ loading increases the abundance of Ca^{2+}- and voltage-sensitive large-conductance K^+ channels in the apical membrane of surface cells in rat distal colon. These channels constitute the enhanced macroscopic apical K^+ conductance previously identified in these cells, and are likely to play a critical role in the active K^+ secretory process that typifies this model of colonic K^+ adaptation.

Mammalian colonic epithelia possess mechanisms for active K^+ absorption and K^+ secretion that adapt to chronic changes in dietary K^+ intake, and thereby help to maintain K^+ homeostasis (Binder & Sandle, 1994). These phenomena have been studied in most detail in rat colon, especially in the distal segment, where dietary K^+ depletion enhances active K^+ absorption and dietary K^+ loading reverses basal net K^+ absorption to net K^+ secretion (Foster, Sandle, Hayslett & Binder, 1986). Transformation of the K^+ transport properties of the distal colon during dietary K^+ loading reflects increased K^+ uptake across an amplified basolateral membrane containing a greater number of Na^+–K^+-ATPase units (and presumably, Na^+–K^+–$2Cl^-$ cotransport sites); a rise in intracellular K^+ concentration; and a considerable increase in the TEA-sensitive apical K^+ conductance of surface cells (Kashgarian, Taylor, Binder & Hayslett, 1980; Sandle, Foster, Lewis, Binder & Hayslett, 1985). These cellular responses to K^+ loading constitute an active K^+ secretory process which requires both the increased dietary K^+ load *per se* and the associated rise in circulating plasma aldosterone concentration (Foster, Jones, Hayslett & Binder, 1985). Furthermore, under voltage clamp conditions, inhibition of the parallel aldosterone-induced electrogenic Na^+ absorptive process by amiloride results in a negative (rather than zero) transepithelial short-circuit current which equates with the net K^+ secretory flux (Foster *et al.* 1986), indicating the underlying electrogenicity of the K^+ secretory process.

Although the above studies have provided considerable insights into the cellular basis for the changes in K^+ transport that occur in rat distal colon during dietary K^+ loading, the nature of the enhanced apical K^+ conductance has not been explored at the single channel level. Previous studies have highlighted the difficulties in single channel recording from the apical membrane of intact rat distal colon, even after removal of surface mucus (Diener, Rummel, Mestres & Lindemann, 1989). Nevertheless, large-

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conductance (120–150 pS) K^+ channels have been identified in a small number of cell-attached apical membrane patches on surface cells in intact distal colonic mucosa from control animals (Diener *et al.* 1989). In the present study, single channel recordings were obtained from the plasma membrane of isolated surface cells and the apical membrane of surface cells around the openings of intact crypts from the distal colon of control and dietary K^+-loaded rats. Our data show that dietary K^+ enrichment stimulates a considerable increase in the abundance of large-conductance apical K^+ channels. These K^+ channels, which are Ca^{2+} sensitive, voltage sensitive at 'intracellular' concentrations of free Ca^{2+}, and inhibited by quinidine, TEA and Ba^{2+}, are likely to play an important part in the electrogenic K^+ secretory process induced in rat distal colon by dietary K^+ loading.

METHODS

Preparation of animals

Experiments were performed in adult male Sprague–Dawley rats (200–300 g). Control animals were fed 20 g per day of normal rat chow (daily dietary K^+ intake, 1·8 mmol). Dietary K^+-loaded animals were fed for 10–14 days with 20 g per day of chow enriched with KCl (daily dietary K^+ intake, 14·4 mmol). A control or K^+-loaded animal was chosen at random (by G.I.S.) on the day of the experiment, killed by cervical dislocation, and the distal 5 cm of colon removed and placed in ice-cold (0–4 °C) 0·9% NaCl solution. Subsequent surface cell/crypt isolation procedures and patch clamp recordings were undertaken (by I.B.) without prior knowledge of the K^+ status of the animals.

Isolation of surface cells from distal colon

Surface cells were isolated from the distal colon using a method described previously (Steiger, Marxer & Hauri, 1986). The distal colonic segment was flushed gently five times with 10 ml of ice-cold 0·9% NaCl solution containing 10 mM D-glucose and 0·5 mM dithiothreitol (DTT), and opened longitudinally to expose the mucosal surface. The tissue was placed in 60 ml of isolation buffer containing (mM): Na^+, 40; Cl^-, 30; EDTA, 5; DTT, 0·5; Hepes, 8; buffered to pH 7·6 with Tris, and kept at room temperature for 45 min with gentle shaking for 30 s at 10 min intervals, after which the tissue was removed from the cell suspension. Cells were harvested by centrifugation (2000 r.p.m. for 5 min; Mistral 2000 swing-out rotor), and the pellet resuspended in 30 ml of the K_2SO_4 solution (see below), washed and recentrifuged (2000 r.p.m. for 5 min). This was repeated five times and the final pellet resuspended in 5 ml of K_2SO_4 solution and kept on ice.

Isolation of intact crypts from distal colon

The distal colonic segment was flushed three times with 10 ml of a NaCl Ringer-like solution containing (mM): Na^+, 146; K^+, 4·2; Ca^{2+}, 1·2; Mg^{2+}, 1·2; Cl^-, 125·8; HCO_3^-, 1·6; HPO_4^{2-}, 1·2; $H_2PO_4^-$, 0·2; D-glucose, 5; buffered to pH 7·4 by gassing with 95% O_2–5% CO_2. The segment was threaded over a glass rod and secured at both ends with cotton thread. The serosa and muscle layers were stripped away, the segment everted over the rod and resecured. The glass rod–colonic segment assembly was connected to a vibrator, and incubated for 30 min in the NaCl Ringer-like solution containing 5 mM EDTA and 0·5 mM DTT (gassed with 95% O_2–5% CO_2), during which the assembly was vibrated for 30 s at 60 Hz every 5 min. At the end of the incubation period, the colon was vibrated for a further 60 s whilst being physically agitated with the polished tip of a glass Pasteur pipette, which released intact crypts from the basement membrane. Crypts were collected by centrifugation, the pellet resuspended in 25 ml of NaCl Ringer-like solution and washed five times (centrifugation at 1200 r.p.m. for 5 min throughout). The pellet was finally resuspended in 5 ml of NaCl Ringer-like solution and kept on ice.

Single channel recording techniques

A stock solution of 50 μl ml^{-1} polyethylenimine in 0·5 M sodium borate (pH 8·3) was diluted 1 : 100 with the borate solution, and plastic coverslips (Thermanox, Nunc, Inc., Naperville, IL, USA) were soaked in the solution for 1–2 h. Coverslips were then washed thoroughly with distilled water, and an aliquot of the surface cell or crypt suspension (kept on ice) placed on a coated coverslip and left for 10 min to allow adherence of cells or crypts to the coverslip surface. The coverslip was placed in a small chamber (volume, 1·5 ml) and viewed on the stage of an Olympus CK inverted microscope with conventional illumination (×400 magnification). The microscope was mounted on a vibration isolation table. Single channel recordings were obtained from membrane patches in cell-attached and excised inside-out configurations as described previously (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Patch pipettes were fabricated from fibre-filled borosilicate capillary tubing (o.d., 1·5 mm; i.d., 0·86 mm; Clark Electromedical Instruments) using a two-stage vertical puller (PP-83, Narishige), and the tips fire polished using a microforge (MF-83, Narishige). In some experiments, pipette tips were filled with K_2SO_4 solution containing (mM): K^+, 145; Ca^{2+}, 1·2; Mg^{2+}, 1·2; Cl^-, 5; SO_4^{2-}, 72·3; D-glucose, 10; Hepes, 10; titrated to pH 7·4 with KOH; the chamber (bath) was filled with a Na_2SO_4 solution containing (mM): Na^+, 140; K^+, 4·5; Ca^{2+}, 1·2; Mg^{2+}, 1·2; Cl^-, 5; SO_4^{2-}, 72·3; D-glucose, 10; Hepes, 10; titrated to pH 7·4 with NaOH. In other experiments, the pipette and the bath were filled with K_2SO_4 solution, which depolarized the cells, allowing K^+ currents to be measured at the command voltage (V_{com}) applied to the pipette via the patch clamp amplifier (EPC-7, List Electronics). Pipette and membrane seal resistances were 4–15 MΩ and 4–30 GΩ, respectively. The pipette and bath electrodes were silver chloride-coated silver wires. Experiments were performed at room temperature (20–22 °C).

Single channel currents were recorded with the patch clamp amplifier and stored on videotape after pulse-code modulation (PCM 701ES, Sony). Stored currents were low-pass filtered at 750 Hz, and loaded into computer memory (Elonex PC 386S-200) via a DigiData 1200 interface system (Axon Instruments) at a sampling frequency of 2·5 kHz using pCLAMP software (version 5.1, Axon Instruments). Voltages applied to the membrane patches (V_{com}) were referenced to the interior of the patch pipette. Current–voltage (I–V_{com}) relations were constructed by plotting the single channel current (equal to the difference between the open and closed channel current) at each value of V_{com}. Goldman–Hodgkin–Katz (GHK) current and voltage equations (Goldman, 1943; Hodgkin & Katz, 1949) were used to calculate $K^+ : Na^+$ permeability ($P_K : P_{Na}$) ratios and reversal potentials (V_{rev}), respectively. Single channel open probability was determined using an analysis program written in Quick Basic 4.0 (Microsoft). Transitions between the fully closed and fully open current levels occurred when the current crossed a threshold set midway between these two states. Single channel open probability (P_o) was calculated as:

$$P_o = (\Sigma n t_n)/N,$$

where N is the maximum number of channels seen to be open simultaneously during 30 s of recording under a specific set of

experimental conditions (verified by the number of peaks on current amplitude histograms generated during single channel analyses), n represents the state of the channels (0, closed; 1, one channel open, etc.) and t_n is the time spent in state n.

The regulation of K^+ channels by Ca^{2+} was studied in excised inside-out patches of isolated surface cells from the distal colon of K^+-loaded animals, with K_2SO_4 solution (containing 1·2 mM Ca^{2+}) in the pipette and K_2SO_4 solution in the bath containing varying amounts of Ca^{2+} buffered with 5 mM EGTA to provide free Ca^{2+} concentrations of 1 nM, 10 nM, 32 nM, 100 nM and 1 μM (Fabiato, 1988).

The effects of the K^+ channel blockers quinidine (1 mM) and TEA (20 mM) were evaluated in excised inside-out patches of isolated distal colonic surface cells from K^+-loaded animals with K_2SO_4 solution (containing 1·2 mM Ca^{2+}) in the pipette and the bath. In order to study K^+ channel blockade by Ba^{2+} (5 mM), both the pipette and the bath were filled with a KCl solution containing (mM): K^+, 145; Ca^{2+}, 1·2; Mg^{2+}, 1·2; Cl^-, 149·8; D-glucose, 10; Hepes, 10; titrated to pH 7·4 with KOH.

Results are generally expressed as means ± S.E.M., and comparisons between mean values were made using a two-tailed Student's t test for paired data. The frequency distributions of K^+ channels in patches on isolated surface cells from control and K^+-loaded animals were compared using a two-tailed Mann–Whitney U test. The data were also modelled as a Poisson distribution in order to estimate the overall increase in K^+ channel density in response to dietary K^+ loading. In all instances, $P < 0·05$ was considered significant.

RESULTS

Effect of K^+ loading on abundance of large-conductance channels

Isolated surface cells

In view of the technical difficulties encountered in recording apical ion channel activity in intact sheets of rat distal colonic epithelium, no attempt was made to patch the apical membrane of surface colonocytes in this type of preparation, and we elected to evaluate the effect of K^+ loading on K^+ channel abundance using isolated surface cells. The success rate for obtaining gigaohm membrane seals was greater than 75%. With K_2SO_4 solution in the pipette and either K_2SO_4 or Na_2SO_4 solution in the bath, large-conductance channels with a high P_o (~0·85) were seen in 9 of 90 (10%) cell-attached patches in surface cells isolated from the distal colon of control animals. However, similar large-conductance channels were present in 247 of 437 (57%) cell-attached patches in surface cells from K^+-loaded animals. As shown in Fig. 1, K^+ loading produced a significant shift in the frequency distribution of the large-conductance channels ($P < 0·001$). In surface cells from control animals, one channel was seen in seven patches, two channels in one patch, and three channels in one patch. By contrast, 45% of patches on surface cells from K^+-loaded animals contained at least two channels, with up to seven channels occurring in some patches. Modelling the data as a Poisson distribution (and correcting for over-dispersion by scaling the errors using the ratio of residual deviance/degrees of freedom), we estimated that dietary K^+ loading increased K^+ channel density by a factor of 11·1 (95% confidence limits, 5·5 and 22·3).

Surface cells *in situ*

Since isolated surface cells were non-polarized, it was unclear whether or not the large-conductance channels originated from the apical membrane. This problem was addressed by recording from the apical membrane of surface cells surrounding the openings of intact crypts isolated from the distal colon of control and K^+-loaded animals, with K_2SO_4 solution in the pipette and the bath. Obtaining gigaohm membrane seals was more difficult in this preparation (20% success rate) than in isolated surface cells, despite additional washing of the crypts with K_2SO_4 solution containing 1 mM DTT, and it was not possible to map the distribution density of these channels around the crypt openings. Nevertheless, whereas a single large-conductance channel was seen in only 1 of 11 (9%) cell-attached apical membrane patches in crypts from control animals, identical channels (up to five per patch) were seen

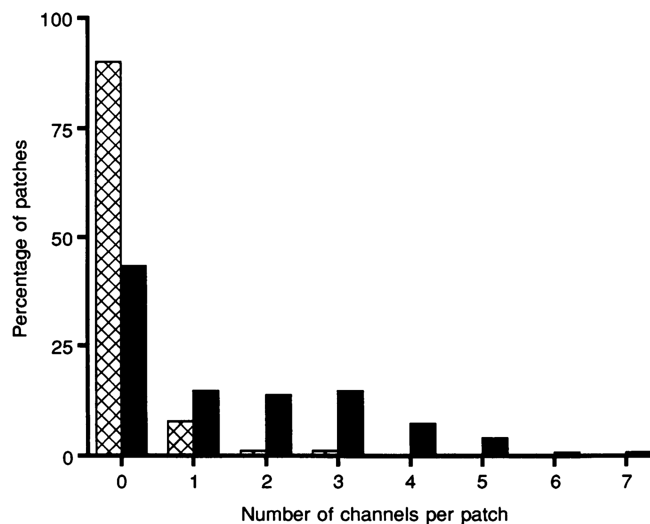

Figure 1. Effect of dietary K^+ loading on the abundance of large-conductance channels in isolated surface cells from rat distal colon

The percentage of patches containing a given number of channels is shown on the y-axis, and the numbers of channels per patch are shown on the x-axis. ▩, control animals (90 patches in total); ■, K^+-loaded animals (437 patches in total). The data from the two groups of animals were significantly different ($P < 0·001$, Mann–Whitney U test).

in 9 of 13 (69%) cell-attached apical membrane patches in crypts from K^+-loaded animals. There were insufficient apical membrane patches on surface cells *in situ* to perform a statistical analysis of the effect of K^+ loading on the frequency distribution of the channels. However, taken together, the data from isolated surface cells and surface cells *in situ* indicate that K^+ loading stimulated a considerable increase in the abundance of large-conductance channels, which reside in the apical membrane.

Basic properties of large-conductance channels

Isolated surface cells

Figure 2*A* shows typical current recordings from a cell-attached patch on an isolated surface cell bathed in Na_2SO_4 solution (K_2SO_4 solution in pipette) with the corresponding linear I–V_{com} relation (Fig. 2*B*). Data from eight such experiments using cells from K^+-loaded animals provided a mean slope conductance and V_{rev} of 139 ± 6 pS and

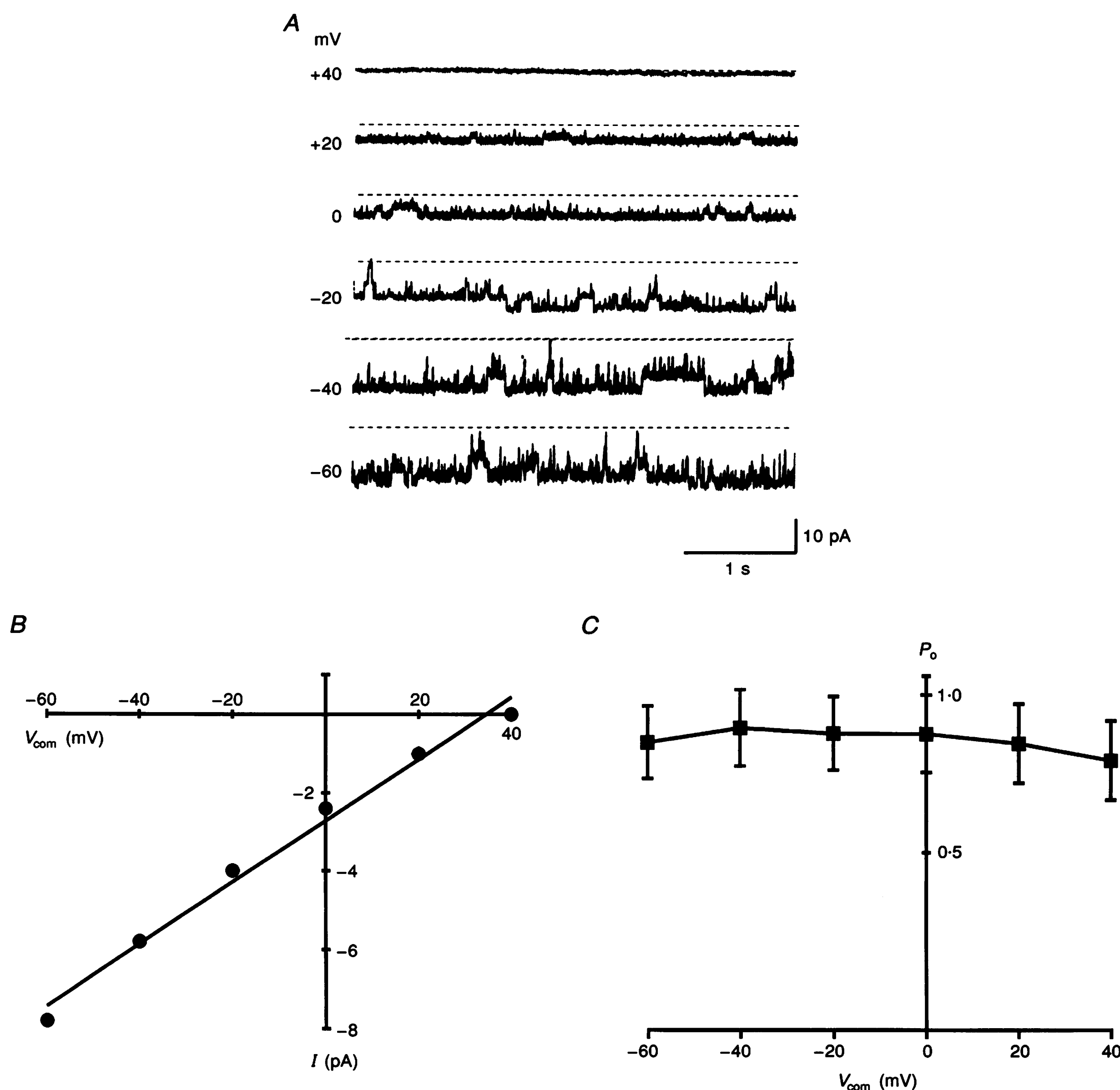

Figure 2. Large-conductance K^+ channels in isolated surface cells from rat distal colon

A shows typical recordings from a cell-attached patch on a surface cell from a K^+-loaded animal at different command potentials (V_{com}) referenced to pipette interior (Na_2SO_4 solution in bath, K_2SO_4 solution in pipette). In this and the other figures, dashed lines indicate zero current levels, downward current deflections indicate K^+ flow from the pipette to the cell or bath, and upward current deflections indicate K^+ flow from the cell or bath to the pipette. *B* shows the I–V_{com} relation of the recordings in *A* (data fitted by linear regression analysis). *C* shows the voltage sensitivity of K^+ channel activity (P_o, single channel open probability; $n = 8$ patches).

$35{\cdot}5 \pm 0{\cdot}9$ mV, respectively. Figure 2C shows that a high level of spontaneous channel activity was maintained when V_{com} was varied between −60 and 40 mV, indicating that the large-conductance channel gated independently of voltage under these experimental conditions. Large-conductance channels with identical properties were seen in cell-attached patches on surface cells isolated from control animals (data not shown).

Figure 3A shows typical current recordings from an excised inside-out patch with Na_2SO_4 solution in the bath and K_2SO_4 solution in pipette, with the corresponding curvilinear I–V_{com} relation (Fig. 3B). Data from seven such experiments provided mean calculated values of $P_K : P_{Na}$ and V_{rev} of $266 \pm 12:1$ and $84{\cdot}9 \pm 0{\cdot}1$ mV, respectively. As shown in Fig. 3C, replacing Na_2SO_4 solution in the bath with K_2SO_4 resulted in a linear I–V_{com} relation, with a mean

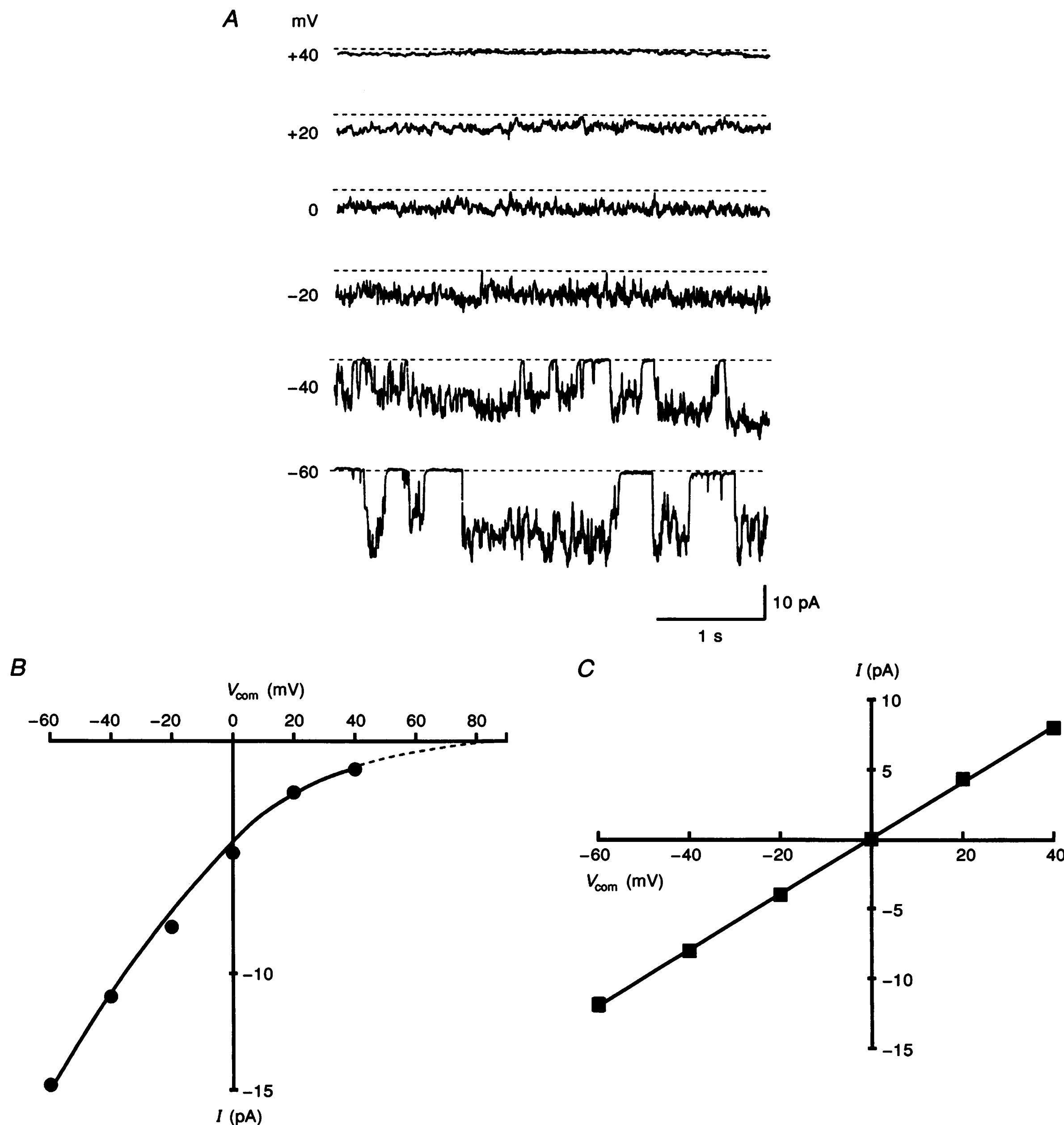

Figure 3. Large-conductance K^+ channels in isolated surface cells from rat distal colon

A shows typical recordings from an inside-out patch of membrane from a surface cell isolated from a K^+-loaded animal at different V_{com} values referenced to the pipette interior (Na_2SO_4 solution in the bath, K_2SO_4 solution in the pipette). B shows the curvilinear I–V_{com} relation of the recordings in A (data fitted and reversal potential obtained using the GHK current and voltage equations). C shows the linear I–V_{com} relation in an inside-out patch with K_2SO_4 solution in the bath and pipette (data fitted by linear regression analysis).

slope conductance of 209 ± 6 pS ($n = 16$). These results indicate that the large-conductance channels were almost perfectly selective for K^+ over Na^+. The level of channel activity in the inside-out patches (irrespective of whether Na_2SO_4 or K_2SO_4 solution was present in the bath) when V_{com} was varied between −60 and 40 mV was identical to that seen in the cell-attached configuration (Fig. 2C).

Surface cells *in situ*

Figure 4A shows typical current recordings from an excised inside-out patch of apical membrane from a surface cell at the opening of a colonic crypt. The patch was bathed symmetrically in K_2SO_4 solution (containing 1·2 mM Ca^{2+}), and the corresponding linear I–V_{com} relation is shown in Fig. 4B. The mean slope conductance was 233 ± 14 pS

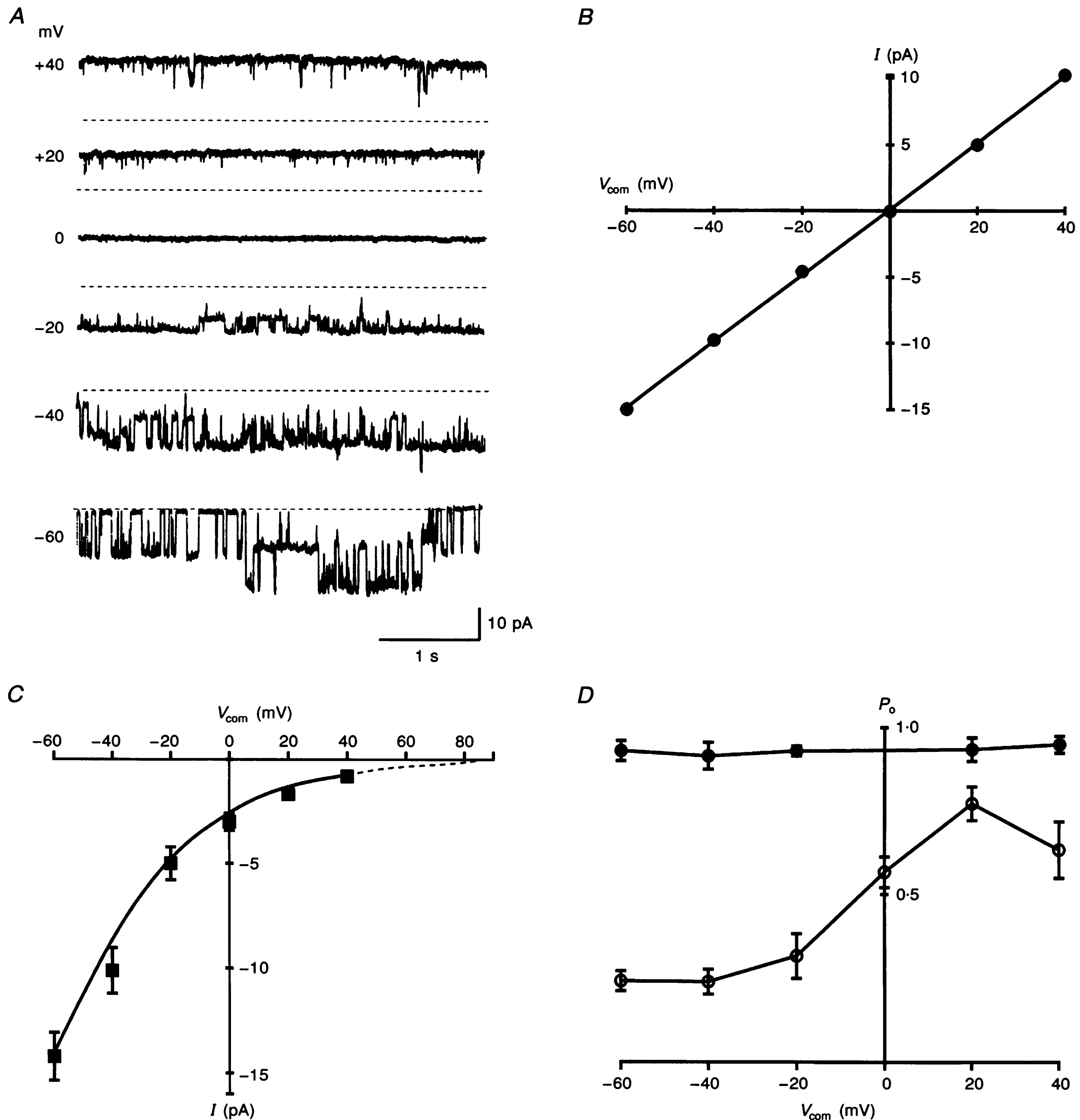

Figure 4. Large-conductance apical K^+ channels in surface cells of rat distal colon

A shows typical recordings from an inside-out patch of apical membrane from a surface cell from a K^+-loaded animal at different V_{com} values referenced to the pipette interior (K_2SO_4 solution in bath and pipette). B shows the linear I–V_{com} relation of the recordings in A (data fitted by linear regression analysis). C shows the curvilinear I–V_{com} relation after substituting Na_2SO_4 for K_2SO_4 in the bath (data fitted and V_{rev} obtained using the GHK current and voltage equations). D shows the voltage sensitivity of K^+ channel activity with K_2SO_4 (●) or Na_2SO_4 (○) present in the bath solution ($n = 3$ patches).

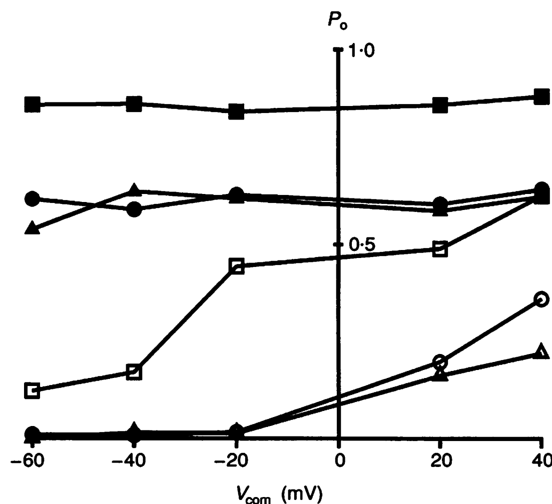

Figure 5. Effect of Ca^{2+} and voltage on large-conductance K^+ channel activity in isolated surface cells from rat distal colon

P_o was determined over a range of V_{com} values using inside-out patches from surface cells isolated from the distal colon of K^+-loaded animals (K_2SO_4 solution containing 1·2 mM in pipette, and K_2SO_4 solution containing varying concentrations of free Ca^{2+} in the bath). Data at Ca^{2+} concentrations of 1 nM (△), 10 nM (○), 100 nM (▲) and 1 μM (●) were obtained from 4 patches, those at 32 nM (□) from 24 patches, and those at 1·2 mM (■) from 25 patches. Error bars omitted for clarity.

($n = 3$). The K^+ selectivity of this channel was explored by replacing the bath solution with Na_2SO_4 and determining the effect on the I–V_{com} relation of the channel. As shown in Fig. 4*C*, substituting Na^+ for K^+ in the bath solution shifted V_{rev} from 0 mV to a calculated value of 84·7 mV, indicating a $P_K : P_{Na}$ ratio of 247 : 1, and the mean values from three patches were 84·7 ± 0·2 mV and 249 ± 15 : 1, respectively. Figure 4*D* shows that the high levels of channel activity appeared to be voltage insensitive when K_2SO_4 (containing 1·2 mM Ca^{2+}) was present in both the bath and the pipette. By contrast, channel activity was generally less with Na_2SO_4 solution (containing 1·2 mM Ca^{2+}) in the bath, but under these conditions, channel activity increased 2- to 3-fold when the membrane was depolarized from −60 to 40 mV.

Sensitivity to Ca^{2+} and voltage

As described above, a high level of voltage-insensitive K^+ channel activity (P_o, ~0·85) was observed consistently in excised inside-out patches when bathed in symmetrical K_2SO_4 solution containing 1·2 mM Ca^{2+}. The role of 'cytosolic' Ca^{2+} in regulating K^+ channel activity was studied in greater detail in excised inside-out patches ($n = 4$) of isolated surface cells from the distal colon of K^+-loaded animals. After excision, patches were bathed in EGTA-containing K_2SO_4 solutions (pH maintained at 7·4 with KOH) containing concentrations of free Ca^{2+} in the sequence 1 nM, 10 nM, 32 nM, 100 nM and 1 μM. Data were also obtained from twenty additional patches bathed in K_2SO_4 solution containing 32 nM Ca^{2+}. Figure 5 summarizes the level of channel activity at each concentration of bath Ca^{2+} (pipette contained K_2SO_4 solution with 1·2 mM Ca^{2+} throughout) over a range of command voltages, and data obtained with 1·2 mM Ca^{2+} in the bath ($n = 25$) are included for comparison. At 1 nM Ca^{2+}, channel activity was low at −40 mV (P_o, 0·014 ± 0·012), but increased 16-fold after depolarization to 40 mV (P_o, 0·219 ± 0·076). Increasing bath Ca^{2+} to 10 nM and then 32 nM produced a progressive increase in channel activity at all voltages, while the stimulatory effect of depolarization on channel activity was retained. Although P_o was generally greater when bath Ca^{2+} was raised to 100 nM, the influence of voltage at this Ca^{2+} concentration was negligible, and changing bath Ca^{2+} to 1 μM had no further significant effect on P_o. Channel activity was at its highest level with 1·2 mM Ca^{2+} in the bath. In summary, these data indicate that the large-conductance apical K^+ channel stimulated during dietary K^+ loading is both Ca^{2+} and voltage sensitive at 'cytosolic' Ca^{2+} concentrations below 100 nM.

Effects of K^+ channel blockers

The effects of the classic K^+ channel blockers quinidine, TEA and Ba^{2+} were tested in excised inside-out patches of isolated surface cells from distal colon of K^+-loaded animals. In the experiments with quinidine and TEA, patches were bathed symmetrically in K_2SO_4 solution containing 1·2 mM

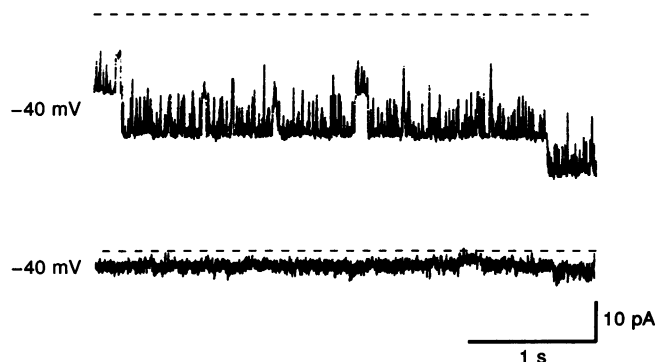

Figure 6. Effect of quinidine on large-conductance K^+ channels

Typical recordings from an inside-out patch of membrane from a surface cell isolated from the distal colon of a K^+-loaded animal ($V_{com} = -40$ mV). K_2SO_4 solution was present in both the bath and the pipette. Upper recording obtained before, and lower recording obtained after, the addition of 1 mM quinidine to the bath. Unitary currents were unchanged by quinidine.

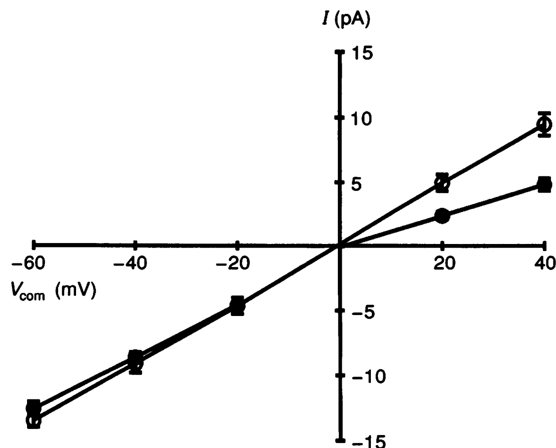

Figure 7. Effect of TEA on large-conductance K^+ channels

I–V_{com} relations of large-conductance K^+ channels in inside-out patches ($n = 4$) from surface cells isolated from the distal colon of K^+-loaded animals. K_2SO_4 solution present in both the bath and the pipette. Data points are mean values (±S.E.M.) before (○) and after (●) the addition of 20 mM TEA to the bath. TEA reduced single channel conductance by 50% at depolarizing voltages, while P_o was unchanged across the entire voltage range.

Ca^{2+}. Symmetrical KCl solutions were used to study the effect of Ba^{2+}.

Quinidine

As shown in Fig. 6, addition of 1 mM quinidine to the bath reduced channel activity in 6 of 6 patches, P_o decreasing from 0·896 ± 0·022 to 0·006 ± 0·001 ($P < 0·001$) at a value for V_{com} of −40 mV, but there was no change in unitary current.

TEA

In contrast to quinidine, addition of 20 mM TEA to the bath had no effect on P_o at V_{com} of −40 mV (0·894 ± 0·024 pre-TEA *versus* 0·898 ± 0·015 post-TEA) or 40 mV (0·923 ± 0·052 pre-TEA *versus* 0·961 ± 0·012 post-TEA). However, Fig. 7 shows that whereas TEA had no effect on unitary current amplitude at hyperpolarizing voltages, unitary currents were reduced by 50% at depolarizing voltages in 4 of 4 patches. These data indicate that TEA produced 'fast'-type channel blockade at depolarizing voltages when added to the 'cytosolic' face of the cell membrane. It seems likely that under these conditions, TEA blocked and dissociated from the K^+ channel with kinetics that were too rapid to be detected by our recording system, resulting in a 50% decrease in the apparent single channel conductance. Studies in other epithelia indicate that it is not possible to predict the pattern of K^+ channel blockade when TEA is added to the 'cytosolic' face of inside-out patches. TEA decreases the P_o of 85–99 pS Ca^{2+}-insensitive basolateral K^+ channels in rat duodenal crypts (McNicholas, Fraser & Sandle, 1994), 138 pS Ca^{2+}-sensitive basolateral K^+ channels in human colonic crypts (Lomax, Warhurst & Sandle, 1996), and 210–302 pS Ca^{2+}-sensitive basolateral K^+ channels in human respiratory epithelial cells (Kunzelmann, Pavenstädt & Greger, 1989). By contrast,

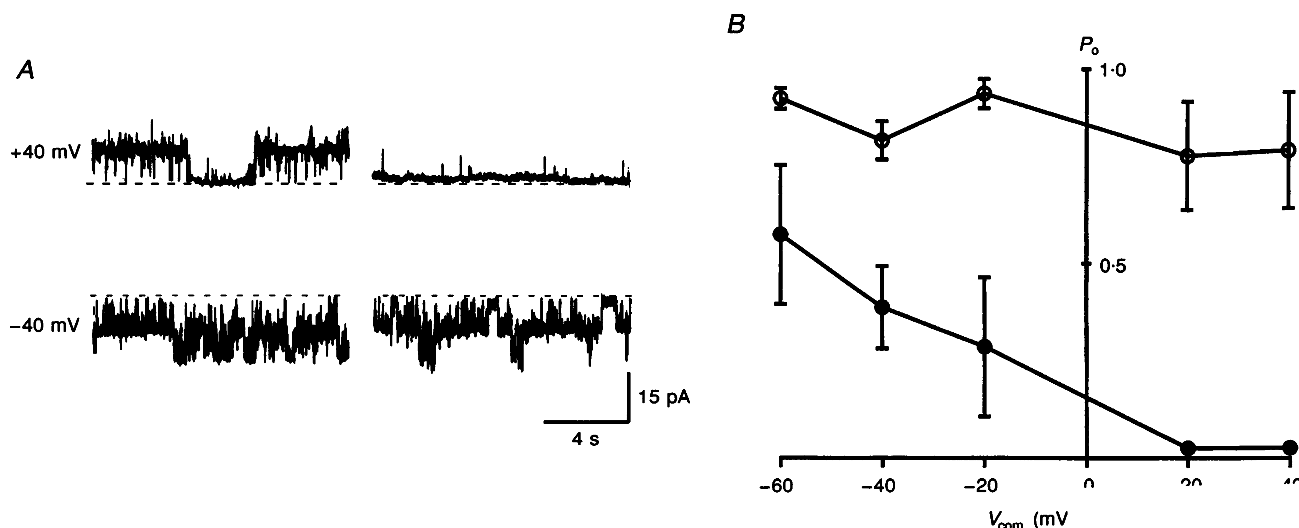

Figure 8. Effect of Ba^{2+} on large-conductance K^+ channels

A shows K^+ channel activity at values for V_{com} of +40 and −40 mV (referenced to pipette interior, KCl solution in the bath and pipette) in an inside-out patch of membrane from a surface cell isolated from a K^+-loaded animal before (traces on left) and after (traces on right) addition of 5 mM Ba^{2+} to the bath. *B* emphasizes the voltage dependency of Ba^{2+} blockade of K^+ channel activity. Data points were obtained from 3 patches before (○) and after (●) addition of 5 mM Ba^{2+}.

TEA decreases the apparent single channel conductance without changing P_o in 12 pS Ca^{2+}-sensitive K^+ channels in cultured mammary epithelial cells (Furuya, Enomoto, Furuya, Yamagishi, Edwards & Oka, 1989), and 200 pS Ca^{2+}-sensitive K^+ channels in smooth muscle cell membranes (Benham, Bolton, Lang & Takewaki, 1985). In the present study, outside-out patches were not used to test the effects of TEA on the external face of the cell membrane.

Ba^{2+}

As shown in Fig. 8, addition of 5 mM Ba^{2+} to the bath produced voltage-dependent channel blockade in 3 of 3 patches, P_o decreasing from 0.816 ± 0.049 to 0.387 ± 0.106 ($P < 0.01$) at $V_{com} = -40$ mV, and from 0.792 ± 0.149 to 0.026 ± 0.002 ($P < 0.01$) at $V_{com} = 40$ mV, without any change in unitary current.

DISCUSSION

The distal colon of rats fed a normal diet exhibits net K^+ absorption, owing to the presence of an electroneutral K^+–H^+-ATPase-mediated process in the apical membrane of surface cells, which normally has a relatively low K^+ conductance (Sandle & McGlone, 1987; del Castillo, Rajendran & Binder, 1991). Chronic dietary K^+ loading stimulates an electrogenic K^+ secretory process which reflects increased basolateral K^+ uptake across an amplified basolateral membrane, a rise in intracellular K^+ concentration, and an increase in the macroscopic K^+ conductance of the apical membrane (Kashgarian *et al.* 1980; Sandle *et al.* 1985). These intracellular changes combine to produce a 3-fold increase in the unidirectional K^+ flux from serosa to mucosa (together with a 60% decrease in K^+ flux from mucosa to serosa), which results in a reversal of net K^+ absorption to net K^+ secretion (Foster *et al.* 1985, 1986). The full adaptive response to dietary K^+ loading requires a significant degree of secondary hyperaldosteronism in addition to the dietary K^+ load itself (Foster *et al.* 1985). The increase in circulating aldosterone concentration is probably critical for the increase in apical K^+ conductance, since dietary Na^+ depletion (which induces an even greater degree of secondary hyperaldosteronism) elicits a similar net K^+ secretory response which involves the stimulation of a TEA- and Ba^{2+}-inhibitable apical K^+ conductance located mainly in surface cells of the epithelium (Sweiry & Binder, 1989). Furthermore, net K^+ secretion across the distal colon of Na^+ depleted animals is not dependent on Na^+ absorption, since net and unidirectional K^+ fluxes are not changed after the mucosal addition of 0·1 mM amiloride, which inhibits aldosterone-induced apical Na^+ channels and the sole (electrogenic) Na^+ absorptive process (Foster *et al.* 1986). It can therefore be seen that characterization of the apical K^+ conductance at the single channel level is central to understanding the K^+ secretory process induced by dietary K^+ loading and Na^+ depletion.

From our data, we estimate that dietary K^+ loading produced an 11·1-fold increase in K^+ channel density in the plasma membrane of surface cells isolated from the distal colon. It was not possible to perform a similar analysis of the relatively small amount of data obtained from surface cells surrounding the openings of intact distal colonic crypts. Nevertheless, our results suggest that K^+ loading also produces a considerable increase in the abundance of apical K^+ channels in surface cells *in situ.* We have yet to determine whether dietary K^+ loading has a similar effect on apical K^+ channels along the entire surface cell–crypt cell axis. However, this seems unlikely based on the results of studies with site-directed intracellular microelectrodes, which showed that hyperaldosteronism secondary to dietary Na^+ depletion stimulated a TEA-blockable apical K^+ conductance in the K^+ secreting distal colon, which was localized to surface cells and almost absent from upper crypt cells (Lomax, McNicholas, Lombès & Sandle, 1994).

Our results show that K^+ channel activity was dependent on both 'cytosolic' Ca^{2+} concentration and membrane voltage (activity increasing with depolarization) at concentrations of Ca^{2+} previously reported in mammalian colonic epithelial cells (Dharmsathaphorn & Pandol, 1986; Warhurst, Turnberg, Higgs, Tonge, Grundy & Fogg, 1993). The properties of a wide range of epithelial K^+ channels have recently been summarized (Benos & Sorscher, 1992), but within gastrointestinal epithelia, apical K^+ channels with a linear I–V_{com} relation, large conductance (140 pS) and sensitivities to Ca^{2+} and voltage have only been described in *Triturus* gall-bladder (Maruyama, Matsunaga & Hoshi, 1986). In the case of rat intestinal epithelia, Ca^{2+}- and voltage-dependent 250 pS K^+ channels have been identified in the basolateral membrane of small intestinal enterocytes (Morris, Gallacher & Lee, 1986), and the basolateral membrane of rat distal colonic crypt cells has been shown to contain Ca^{2+}-dependent, Ba^{2+}- and TEA-inhibitable 12 pS K^+ channels (seen frequently), Ca^{2+}-dependent and Ba^{2+}-inhibitable 187 pS K^+ channels (seen infrequently), and clusters of 27 pS non-selective cation channels (Siemer & Gögelein, 1992; Burckhardt & Gögelein, 1992). Other large-conductance K^+ channels exist in the basolateral membrane of crypt cells in rabbit and human distal colon (Loo & Kaunitz, 1989; Lomax *et al.* 1996). Our study, however, appears to be the first to have characterized a functionally important *apical* K^+ channel in mammalian colon.

The localization of these large-conductance K^+ channels to the apical membrane of distal colonic surface cells, and their increased abundance during dietary K^+ loading, provides strong evidence that they have a pivotal role in the active K^+ secretory process that typifies this model of colonic K^+ adaptation. It is now clear that these K^+ channels constitute the TEA-inhibitable apical K^+ conductance induced by dietary K^+ loading in rat distal colon, which has been identified in previous microelectrode studies (Sandle *et al.* 1985). It also seems likely that they account for the increase in TEA- and Ba^{2+}-inhibitable apical K^+ conductance that occurs predominantly in surface cells of rat distal colon during dietary Na^+ depletion (Sweiry & Binder, 1989; Lomax

et al. 1994). Although the large-conductance K^+ channels are voltage sensitive at 'cytosolic' Ca^{2+} concentrations, we predict from the data shown in Fig. 5 that hyperpolarization of the apical membrane *in vivo* (produced, for example, by amiloride blockade of co-existent apical Na^+ channels) should have only a small effect on P_o – and therefore net K^+ secretion – at intracellular Ca^{2+} concentrations of 100 nM or above. This hypothesis is supported by perfusion studies in the distal colon of control and Na^+-depleted rats, which showed that intraluminal amiloride abolished the transmucosal potential difference and decreased the plasma to lumen (secretory) K^+ flux in the Na^+-depleted group, which nevertheless remained considerably higher than in the control group, consistent with the presence of an aldosterone-induced, potential-independent, transcellular K^+ secretory pathway (Edmonds, 1981). In addition, while human proximal rectum possesses a Ba^{2+}-inhibitable apical K^+ conductance (Goldstein, Shapiro, Rao & Layden, 1991), intraluminal amiloride had no effect on net K^+ secretion despite reducing net Na^+ absorption and transmucosal potential difference (Sandle, Gaiger, Tapster & Goodship, 1986).

Although dietary K^+ loading increased the number of functional apical K^+ channels in surface cells of rat distal colon, the cellular basis for this change remains unclear. Complete reversal of net K^+ absorption to net K^+ secretion by dietary K^+ loading certainly requires both the increased dietary K^+ load and a significant degree of secondary hyperaldosteronism (Foster *et al.* 1985). However, the increase in dietary K^+ load *per se* may be sufficient to stimulate the expression of apical K^+ channels in surface cells of rat distal colon, since dietary K^+ enrichment increases the macroscopic K^+ conductance and the K^+ currents flowing across the apical membrane of renal cortical collecting duct cells in adrenalectomized rabbits (Muto, Sansom & Giebisch, 1988). Further studies are required to determine whether the increases in dietary K^+ load and/or levels of circulating aldosterone stimulate the synthesis of new K^+ channel protein(s) or the recruitment of pre-existing K^+ channels from within the cytosol. In any event, maximal levels of apical K^+ channel activity may also depend on aldosterone-mediated changes in other intracellular regulatory mechanisms dependent on Ca^{2+} concentration and pH. Although intracellular Ca^{2+} concentrations were not measured in this study, the Ca^{2+}-mediated cholinergic muscarinic agonist bethanechol stimulates active K^+ secretion in rat proximal colon by enhancing the serosal to mucosal K^+ flux, a change which may involve the activation of Ca^{2+}-sensitive apical K^+ channels (Foster, Sandle, Hayslett & Binder, 1983). In addition, aldosterone stimulates electrogenic Na^+ transport (and by inference, apical Na^+ conductance) in A6 renal cells after a delay of 60 min, an effect dependent on a 3-fold transient rise in intracellular Ca^{2+} concentration (Petzel *et al.* 1992). Preliminary studies with excised inside-out patches have shown that the large-conductance K^+ channels are also stimulated by increasing the bath pH from 7·4 to 7·6, and that dietary K^+ loading is associated with intracellular alkalinization (by 0·26 pH units) of distal colonic surface cells (Butterfield & Sandle, 1995). It remains to be seen whether the rise in intracellular pH has a direct causal role in increasing apical K^+ channel activity during dietary K^+ loading, or whether such a mechanism reflects aldosterone-mediated stimulation of Na^+–H^+ exchange, as reported in renal tubular cells (Oberleithner, Kersting & Hunter, 1988).

In summary, we have shown that dietary K^+ loading increases the abundance of large-conductance K^+ channels in the apical membrane of surface cells in rat distal colon. These channels are Ca^{2+}, voltage and pH sensitive, and are likely to play an important part in the active K^+ secretory process stimulated during dietary K^+ loading. However, the precise interaction of these factors in modulating apical K^+ channels, and whether dietary K^+ loading stimulates similar K^+ channels in the proximal colon and at other points along the surface cell–crypt cell axis, remains to be established.

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