# Potassium secretion in rat distal colon during dietary potassium loading: role of pH regulated apical potassium channels

G I Sandle, I Butterfield

**Abstract**

***Background*—Chronic dietary $K^+$ loading increases the abundance of large conductance (210 pS) apical $K^+$ channels in surface cells of rat distal colon, resulting in enhanced $K^+$ secretion in this epithelium. However, the factors involved in the regulation of these $K^+$ channels are at present unclear.**

***Aims*—To evaluate the effect of dietary $K^+$ loading on intracellular pH and its relation to large conductance apical $K^+$ channel activity in surface cells of rat distal colon.**

***Methods/Results*—As assessed by fluorescent imaging, intracellular pH was higher in $K^+$ loaded animals (7.48 (0.09)) than in controls (7.07 (0.04); $p<0.01$) when surface cells were bathed in NaCl solution, and a similar difference in intracellular pH was observed when cells were bathed in $Na_2SO_4$ solution (7.67 (0.09) and 6.92 (0.05) respectively; $p<0.001$). Ethylisopropylamiloride (EIPA; an inhibitor of $Na^+$-$H^+$ exchange; 1 μM) decreased intracellular pH when surface cells from $K^+$ loaded animals were bathed in either solution, although the decrease was greater when the solution contained NaCl (ΔpH 0.50 (0.03)) rather than $Na_2SO_4$ (ΔpH 0.18 (0.02); $p<0.05$). In contrast, EIPA had no effect in cells from control animals. As assessed by patch clamp recording techniques, the activity of large conductance $K^+$ channels in excised inside-out membrane patches from distal colonic surface cells of $K^+$ loaded animals increased twofold when the bath pH was raised from 7.40 to 7.60. As assessed by cell attached patches in distal colonic surface cells from $K^+$ loaded animals, the addition of 1 μM EIPA decreased $K^+$ channel activity by 50%, consistent with reversal of $Na^+$-$H^+$ exchange mediated intracellular alkalinisation.**

***Conclusion*—Intracellular alkalinisation stimulates pH sensitive large conductance apical $K^+$ channels in rat distal colonic surface cells as part of the $K^+$ secretory response to chronic dietary $K^+$ loading. Intracellular alkalinisation seems to reflect an increase in EIPA sensitive $Na^+$-$H^+$ exchange, which may be a manifestation of the secondary hyperaldosteronism associated with this model of colonic $K^+$ adaptation.**

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$K^+$ is an important yet neglected dietary component. Western diets have a variable $K^+$ content, but a recent survey has indicated average daily $K^+$ intakes in British men and women of 82 and 62 mmol respectively.[1] The habitual dietary intake of large amounts of $K^+$ rich food does not lead to hyperkalaemia or excessive $K^+$ retention in tissues. This is because stimulation of $K^+$ secretory processes in distal nephron segments results in increased urinary $K^+$ excretion,[2] and the ability of chronic dietary $K^+$ loading to stimulate active $K^+$ secretion in rat colon[3] raises the possibility that a similar response may occur in human colon. Although renal $K^+$ homoeostasis becomes impaired during progressive renal insufficiency, many patients remain normokalaemic for long periods without modifying their dietary $K^+$ intake. This reflects, at least in part, the ability of the distal colon to increase its $K^+$ secretory capacity, and a generalised $K^+$ adaptive response throughout the colon may provide an alternative route for $K^+$ excretion as renal $K^+$ excretory function declines.[4 5] This compensatory mechanism could become physiologically significant in otherwise healthy individuals over 60 years of age who have age related but subclinical renal impairment, especially if they adopt a "healthy" low salt diet relatively rich in $K^+$. Despite the potential clinical importance of augmented $K^+$ secretion in the human colon, mechanistic studies are technically difficult, and insights into the underlying cellular processes have evolved entirely from studies in laboratory animals.

In vitro studies have shown that rat distal colon is normally characterised by net $K^+$ absorption, reflecting a ouabain sensitive $K^+$ dependent $H^+$-ATPase located in the apical membrane of surface epithelial cells.[3 6] The distal colonic epithelium increases its capacity for active $K^+$ secretion during dietary $K^+$ loading for 7–10 days.[3] This change in net $K^+$ transport reflects enhanced basolateral $K^+$ uptake (mediated by increased $Na^+$,$K^+$-ATPase and $Na^+$-$K^+$-$2Cl^-$ co-transport activity), a rise in intracellular $K^+$ concentration, and an increase in apical $K^+$ conductance.[3 7 8] We now know that the increase in apical $K^+$ conductance stimulated by dietary $K^+$ loading reflects an 11-fold increase in the abundance of $Ca^{2+}$- and voltage-sensitive 210 pS $K^+$ channels, which are located in the apical membrane of surface epithelial cells.[9] However, it remains unclear whether the increase in apical $K^+$ conductance is a consequence of $K^+$ channel

**Abbreviations used in this paper**: BCECF, 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein: EIPA, ethylisopropylamiloride.

induction (involving de novo synthesis of $K^+$ channel protein), activation of latent $K^+$ channels by one or more intracellular second messengers, or a combination of both possibilities. Complete transition from net $K^+$ absorption to net $K^+$ secretion during dietary $K^+$ loading requires the significant degree of secondary hyperaldosteronism induced by $K^+$ loading as well as the increase in $K^+$ load per se.[10] Interestingly, parallel increases in whole cell $K^+$ conductance and intracellular pH occur in amphibian renal tubular cells following the application of aldosterone, consistent with upregulation of $Na^+$-$H^+$ exchange by the mineralocorticoid hormone.[11] In view of these findings, the aims of the present study were to determine (*a*) whether dietary $K^+$ loading is associated with a $Na^+$-$H^+$ exchange dependent increase in intracellular pH in surface cells of rat distal colon, and (*b*) the role of intracellular pH in regulating the apical $K^+$ channels located in these cells, which are present in greater abundance in this model of $K^+$ adaptation.[9]

## Methods

### PREPARATION OF ANIMALS

Experiments were performed in adult male Sprague-Dawley rats (200–300 g). Control animals were fed 20 g normal rat chow a day (daily dietary $K^+$ intake 1.8 mmol). Dietary $K^+$ loaded animals were fed for 10–14 days with 20 g/day 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. All subsequent experimental manoeuvres were performed blind (by I B).

### ISOLATION OF SURFACE CELLS FROM DISTAL COLON

Surface cells were isolated from the distal colon using a method described previously.[12] The distal colonic segment was flushed gently five times with 10 ml ice cold 0.9% NaCl solution containing 10 mM D-glucose and 0.5 mM dithiothreitol, and opened longitudinally to expose the mucosal surface. The tissue was placed in 60 ml isolation buffer containing (in mM): $Na^+$, 40; $Cl^-$, 30; EDTA, 5; dithiothreitol, 0.5; Hepes, 8; buffered to pH 7.60 with Tris, and kept at room temperature for 45 minutes with gentle shaking for 30 seconds at 10 minute intervals, after which the tissue was removed from the cell suspension. Cells were harvested by centrifugation (2000 rpm for five minutes; Mistral 2000 swing-out rotor), and the pellet resuspended in 30 ml of the $K_2SO_4$ solution (see below), washed and re-centrifuged (2000 rpm for five minutes). This was repeated five times and the final pellet resuspended in 5 ml $K_2SO_4$ solution and kept on ice. Cells were resuspended in $K_2SO_4$ solution to eliminate possible $Na^+$ and $Cl^-$ channel activity, so that large conductance $K^+$ channels could be more easily identified during patch clamp recording in the cell attached configuration. This isolation technique provided predominantly single surface colonocytes with some clumps of cells, which were excluded from intracellular pH measurements and patch clamp recording (see below). Random histological examination of the original distal colonic segments indicated that this technique removed surface cells without releasing the crypts, as seen in a previous study using sodium citrate rather than EDTA to chelate $Ca^{2+}$.[13]

### INTRACELLULAR pH MEASUREMENTS

Single surface cells isolated from the distal colon of the two groups of animals were resuspended in NaCl solution containing (in mM): $Na^+$, 140; $Cl^-$, 149; $K^+$, 4.5; $Ca^{2+}$, 1.2; $Mg^{2+}$, 1.2; D-glucose, 10; Hepes, 10; titrated to pH 7.40 with NaOH. Alternatively, they were resuspended in $Na_2SO_4$ solution containing (in 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 7.40 with NaOH. A stock solution of 50 ml/ml polyethyleneimine in 0.5 M sodium borate (pH 8.30) was diluted 1:100 with the borate solution, and glass coverslips were soaked in the solution for 1–2 h. The coverslips were then washed thoroughly with distilled water. Two or three drops of the cell suspension were placed on a polyethyleneimine coated coverslip, which was then incubated for 10 minutes (room temperature) in 5 ml of the NaCl solution or the $Na_2SO_4$ solution containing 5 μM 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF, a pH sensitive dye). Cells loaded with BCECF were washed three times with 5 ml of the appropriate bathing solution. A coverslip supporting BCECF loaded cells was placed in a small chamber located on the stage of a Nikon Diaphot inverted microscope, and the chamber was perfused continuously with the bathing solution (1ml/min). Cells were excited at 490 and 440 nm, and the fluorescence at these wavelengths recorded on a computer (Apple Macintosh) using Ionvision III software, which was also used for image analysis. Measurements of pH were obtained from about 10 single cells per colon and the mean value was taken as one data point. With both groups of animals, intracellular pH was measured before and two minutes after the addition of 1 μM ethylisopropylamiloride (EIPA) to the bathing solution. This amiloride analogue is a general inhibitor of $Na^+$-$H^+$ exchange isoforms, and has previously been shown to reverse aldosterone stimulated $Na^+$-$H^+$ exchange and the associated rise in intracellular pH in amphibian renal tubular cells.[14]

A pH calibration curve was used to calculate pH values from the 490:440 excitation ratios. Isolated cells were washed in a high $K^+$ buffer containing (in mM): $K^+$, 144.6; $Cl^-$, 146.8; $Ca^{2+}$, 1.2; $Mg^{2+}$, 1.2; $HPO_4^{2-}$, 1.2; $H_2PO_4^-$, 0.2; Hepes, 5; D-glucose, 10. They were then attached to a polyethyleneimine coated coverslip. Cells were loaded with 5 μM BCECF for 10 minutes at room temperature, then rinsed and bathed in the high $K^+$ buffer containing 10 μM nigericin (a proton ionophore) at pH 6.60, 7.00, 7.40, 7.80, and 8.20. After 15 minutes, cells were placed in the chamber and excited at 490 and 440 nm over the pH range to produce a calibration curve.

SINGLE CHANNEL RECORDING TECHNIQUES

An aliquot of the surface cell suspension (kept on ice) was placed on a polyethyleneimine coated plastic coverslip (Thermanox; Nunc, Naperville, Illinois, USA), and left for 10 minutes to allow adherence of cells 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.[15] Patch pipettes were fabricated from fibre filled borosilicate capillary tubing (Clark Electromedical Instruments, Pangbourne, Bucks, UK; outer diameter 1.5 mm, internal diameter 0.86 mm) using a two stage vertical puller (Narishige, Tokyo, Japan; model PP-83), and the tips fire polished using a microforge (Narishige, Tokyo, Japan; model MF-83).

In the first series of patch clamp experiments, the pH sensitivity of 210 pS $K^+$ channels induced in single surface cells by dietary $K^+$ loading was determined using excised inside-out patches of the plasma membrane. Although isolated surface cells were almost certainly non-polarised, we have previously shown that the 210 pS $K^+$ channels are present in the apical membrane of surface cells in situ around the openings of isolated intact rat distal colonic crypts.[9] Thus it seems likely these high conductance $K^+$ channels were distributed throughout the plasma membrane after isolation of the cells, even though they originated from the apical domain. Membrane seals were obtained initially in the cell attached configuration with $K_2SO_4$ solution ((in 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 7.40 with KOH) in both the pipette and the chamber (bath). Virtually $Cl^-$ free solutions were used in all the patch clamp studies to minimise the likelihood of $Cl^-$ channel activity. After excision of the patch into the inside-out configuration, the bath solution was replaced with a similar $K_2SO_4$ solution (pH 7.40, 7.60, or 8.00) containing either 32 nM $Ca^{2+}$ or 200 nM $Ca^{2+}$, buffered with 5 mM EGTA.[16] Channel activity was recorded for 30 seconds over a range of holding voltages (−60 to 40 mV) applied to the pipette via the patch clamp amplifier (List Electronics, Darmstadt, Germany; model EPC-7), and further recordings were obtained after the pH of the bath solution had been changed from 7.40 to 7.60, and then to 8.00. As the concentration of free $Ca^{2+}$ is influenced by the ambient pH, each $K_2SO_4$ solution contained different levels of total $Ca^{2+}$, as previously described.[16]

We then went on to a second series of patch clamp experiments to determine the effect of EIPA on the 210 pS $K^+$ channels induced in surface cells by dietary $K^+$ loading. Cell attached patches were obtained on isolated surface cells bathed in the $Na_2SO_4$ solution, and the pipette was filled with $K_2SO_4$ solution as before. Channel activity was recorded over a range of holding voltages (−60 to 40 mV) before and two minutes after the addition of 1 μM EIPA to the bath solution. EIPA was then washed out of the bath with $Na_2SO_4$ solution, and the recordings repeated.

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 (Sony, Japan; model PCM 701ES). 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, Foster City, California, USA) at a sampling frequency of 2.5 kHz using pClamp software (version 5.1). Voltages applied to the membrane patches ($V_{com}$) were referenced to the interior of the patch pipette. 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 $P_o = (\Sigma nt_n)/N$, where N is the maximum number of channels seen to be open simultaneously during 30 seconds 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.

Results are expressed as mean (SEM), and comparisons between mean values were made using a two tailed Student's *t* test for paired or unpaired data as appropriate. $p<0.05$ was considered significant.

## Results

EFFECT OF DIETARY $K^+$ LOADING ON INTRACELLULAR pH

The effect of dietary $K^+$ loading on the intracellular pH in surface cells was determined with cells bathed in either NaCl solution or $Na_2SO_4$ solution. The intracellular pH of surface cells from $K^+$ loaded animals (7.48 (0.09)) was significantly higher than in control animals (7.07 (0.04), $p<0.01$) when bathed in NaCl solution (n = 4 distal colons in both cases). Intracellular pH was also higher in $K^+$ loaded animals (7.67 (0.09)) than in control animals (6.92 (0.05), $p<0.001$) when cells were bathed in $Na_2SO_4$ solution (n = 4 distal colons in both cases). Although these results suggest that the effect of $K^+$ loading on intracellular pH were more pronounced when $SO_4^{2-}$ ions replaced $Cl^-$ ions in the bathing solution, this difference probably reflects experimental variability rather than being a point of physiological significance (see the Discussion).

EFFECT OF EIPA ON INTRACELLULAR pH

After measuring basal intracellular pH in cells from control and $K^+$ loaded animals, we went

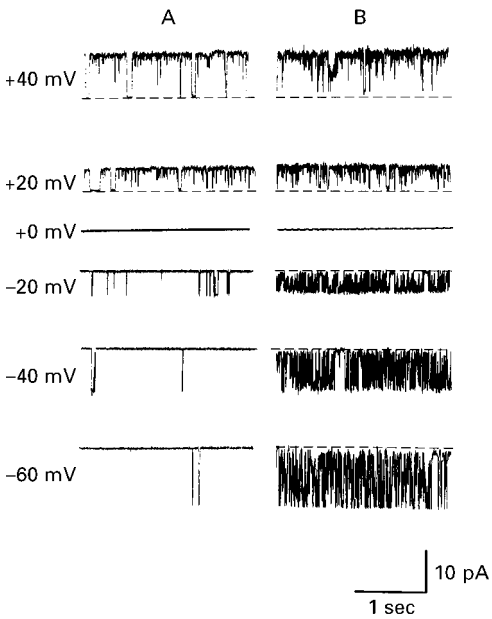


*Figure 1 Effect of pH on large conductance K⁺ channels in isolated surface cells from rat distal colon. Typical recordings from an inside-out patch of membrane from a distal colonic surface cell isolated from K⁺ loaded animals at different $V_{com}$ values, referenced to the pipette interior ($K_2SO_4$ solution containing 32 nM $Ca^{2+}$ in bath, $K_2SO_4$ solution containing 1.2 mM $Ca^{2+}$ in the pipette). (A) and (B) Recordings at bath pH values of 7.40 and 7.60 respectively. The dashed lines indicate zero current levels, upward current deflections indicate K⁺ flow from the bath to the pipette, and downward current deflections indicate K⁺ flow from the pipette to the bath.*

on to determine whether the intracellular alkalinisation induced by $K^+$ loading was related to enhanced $Na^+$-$H^+$ exchange. When cells were bathed in NaCl solution, the addition of 1 μM EIPA had no effect in cells from control animals, whereas intracellular pH decreased from 7.48 (0.09) to 6.98 (0.10) ($p<0.05$) in the $K^+$ loaded animals (n = 4 distal colons in both cases). Similarly, when cells were bathed in $Na_2SO_4$ solution, EIPA had no effect in cells from control animals, but decreased intracellular pH from 7.67 (0.09) to 7.49 (0.02) ($p<0.05$) in the $K^+$ loaded animals (n=4 distal colons in both cases). Furthermore, in cells isolated from $K^+$ loaded animals, EIPA produced a greater decrease in intracellular pH when the bathing solution contained NaCl (ΔpH 0.50 (0.03)) rather than $Na_2SO_4$ (ΔpH 0.18 (0.02), $p<0.05$).

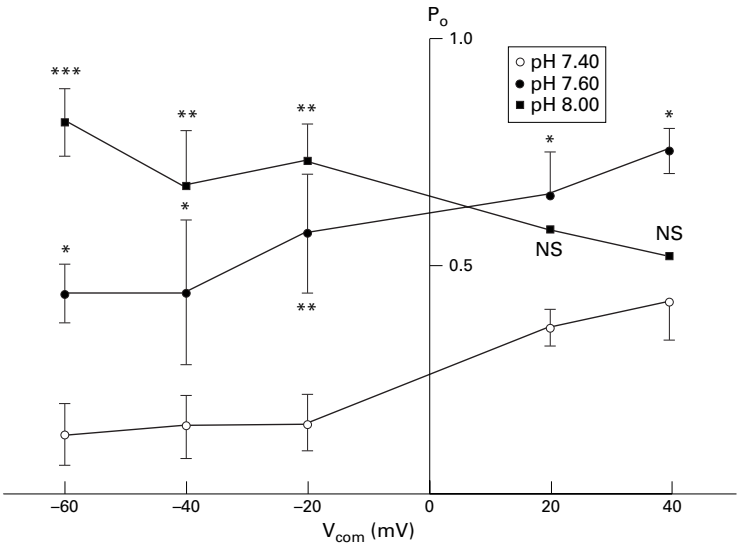


*Figure 2 Effect of pH on large conductance K⁺ channel activity in isolated surface cells from rat distal colon. $P_o$ was determined over a range of $V_{com}$ values using inside-out patches from distal colonic surface cells isolated from K⁺ loaded animals ($K_2SO_4$ solution containing 32 nM $Ca^{2+}$ in bath, $K_2SO_4$ solution containing 1.2 mM $Ca^{2+}$ in the pipette). Data at pH 7.40, 7.60, and 8.00 were obtained from five to eight patches. *p<0.05, **p<0.01, and ***p<0.001 compared with value at pH 7.40. NS, not significant compared with value at 7.40.*

### pH SENSITIVITY OF $K^+$ CHANNELS

Figure 1 shows typical current recordings from an excised inside-out membrane patch from a surface cell isolated from the distal colon of a dietary $K^+$ loaded animal. The patch was bathed symmetrically in $K_2SO_4$ solution (32 nM $Ca^{2+}$ in bath, 1.2 mM $Ca^{2+}$ in pipette). At a bath pH of 7.40, the $K^+$ channel exhibited marked voltage sensitivity, as described previously.[9] Raising the bath pH to 7.60 significantly increased channel activity across the entire voltage range, this effect being most marked at hyperpolarising voltages, although the channel remained voltage sensitive under these conditions. Figure 2 summarises these data (n = 5–8 distal colons) and also shows that channel activity at hyperpolarising voltages increased further at bath pH 8.00, to the extent that the channels were no longer voltage sensitive. When the bath solution contained 200 nM $Ca^{2+}$, the increases in channel activity produced by raising bath pH from 7.40 to 7.60 (data not shown) were similar to those seen when the pH was raised from 7.40 to 7.60 with 32 nM $Ca^{2+}$ in the bath solution. These data indicate that the voltage sensitive 210 pS apical $K^+$ channel induced by dietary $K^+$ loading is particularly pH sensitive over the ranges of membrane voltage and intracellular $Ca^{2+}$ concentration encountered in colonic epithelial cells, although the voltage sensitivity of the channel disappears at excessive levels of alkalinisation (pH 8.00).

### EFFECT OF EIPA ON $K^+$ CHANNEL ACTIVITY

Figure 3A (slow time base) shows a representative experiment (n = 5 distal colons) in which $K^+$ channel activity in a cell attached patch on a distal colonic surface cell from a $K^+$ loaded animal decreased by about 60% within 80 seconds after the addition of 1 μM EIPA to the bath (holding voltage 0 mV). Figures 3B and 3C show sections of the recording in fig 3A on a faster time base. In this experiment, three $K^+$ channels were present in the patch before the addition of EIPA (fig 3B), while only a single open channel level was seen after the addition of EIPA (fig 3C). Figure 3D shows that channel activity was fully restored after EIPA was washed from the bath. Figure 4A summarises the effects of EIPA and its subsequent removal on $K^+$ channel activity in five cell attached patches. The inhibitory effect of EIPA on $K^+$ channel activity was seen over the entire range of holding voltages (fig 4B). These results suggest that stimulation of 210 pS $K^+$ channel activity during dietary $K^+$ loading reflects, at least in part, an increase in intracellular pH which is probably mediated by aldosterone stimulated $Na^+$-$H^+$ exchange.

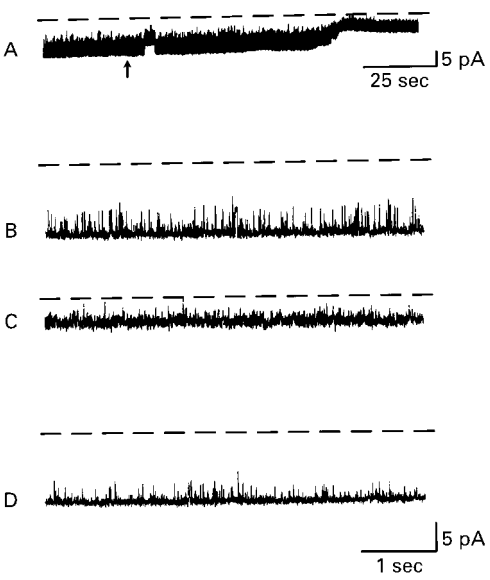


*Figure 3 Effect of 1 μM ethylisopropylamiloride (EIPA) on large conductance $K^+$ channels in isolated surface cells from rat distal colon. Typical recording from a cell attached patch of membrane from a distal colonic surface cell isolated from $K^+$ loaded animal ($Na_2SO_4$ solution in bath, $K_2SO_4$ solution in the pipette; $V_{com}$ = 0 mV). (A) $K^+$ channel activity on slow time base before and after the addition of 1 μM EIPA to the bath (denoted by arrow). Sections of (A) are presented on a faster time base to compare $K^+$ channel activity before the addition of EIPA (B) and afterwards (C). (D) Restoration of channel activity after removal of EIPA. The dashed lines indicate zero current levels, and downward deflections indicate $K^+$ flow from the pipette to the cell.*

## Discussion

Chronic dietary $K^+$ loading increases the apical $K^+$ conductance of surface cells in rat distal colon.[8] This is a major component of the secretory process which transforms net $K^+$ transport from an active absorptive mode to an active secretory mode.[3] This change is dependent on a degree of secondary hyperaldosteronism, as well as the increase in dietary $K^+$ load per se.[10] Although an increased $K^+$ load is itself capable of increasing apical $K^+$ channel activity in renal collecting duct cells from adrenalectomised rabbits,[17] this only changes net $K^+$ absorption to zero net $K^+$ transport in the distal colon of adrenalectomised rats.[10] It is therefore likely that in adrenal-intact rats, the rise in circulating plasma aldosterone concentration produces an additional stimulatory effect on apical $K^+$ channel activity. Recent studies have shown that dietary $K^+$ loading increases the abundance of 210 pS $K^+$ channels located in the apical membrane of surface cells,[9] but it remains unclear whether increases in the dietary $K^+$ load and/or circulating aldosterone levels promote the induction and membrane insertion of new $K^+$ channels, or the activation of "latent" $K^+$ channels already present in the membrane, or a combination of these possibilities. The present study provides new information which indicates that dietary $K^+$ loading produces alkalinisation of surface cells in the distal colon of adrenal-intact animals, and this rise in intracellular pH is a potential stimulant of large conductance (210 pS) apical $K^+$ channels. Although a direct stimulatory effect of aldosterone on apical $K^+$ channel expression/activity cannot be excluded, aldosterone induces parallel increases in intracellular pH and whole cell $K^+$ conductance by upregulating $Na^+$-$H^+$ exchange in amphibian renal tubular cells,[11] and it is more likely that similar events occur in rat distal colonic surface cells during dietary $K^+$ loading.

We did not perform extensive studies of $K^+$ channel pH sensitivity (using inside-out patches) or the effects of EIPA on $K^+$ channel activity (using cell attached patches) in surface cells from control animals because of the inherently low prevalence of channels (roughly 10% of patches) in this group.[9] However, results from an experiment using an inside-out patch from a control animal indicated that the 210 pS $K^+$ channel was pH sensitive, which

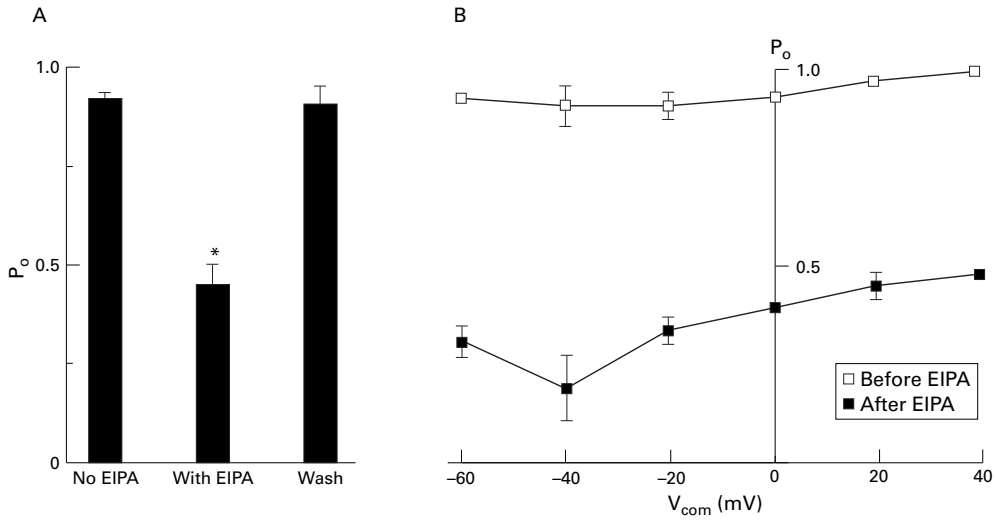


*Figure 4 Effect of 1 μM ethylisopropylamiloride (EIPA) on large conductance $K^+$ channel activity in isolated surface cells from rat distal colon. (A) Summary of results from five cell attached patches of membrane from distal colonic surface cells isolated from $K^+$ loaded animals ($Na_2SO_4$ solution in bath, $K_2SO_4$ solution in the pipette; $V_{com}$ = 0 mV). *p<0.001 compared with the value before the addition of EIPA. (B) Uniform effect of EIPA on $K^+$ channel activity at holding voltages between −60 and 40 mV (n = 3 patches). Error bars not shown were smaller than the symbols. Differences between the values before and after the addition of EIPA were highly significant (p<0.001) at each holding voltage.*

suggests that it does not acquire pH sensitivity during dietary $K^+$ loading. It also seems unlikely that EIPA, which in any case binds externally to $Na^+$-$H^+$ moeities, had a direct inhibitory effect on $K^+$ channel activity in cell attached patches of cells from control animals, in which it had no effect on intracellular pH.

A notable feature of rat distal colon during dietary $K^+$ loading is the persistence of electroneutral $Na^+$ absorption (which reflects apical $Na^+$-$H^+$ and $Cl^-$-$HCO_3^-$ exchanges operating in parallel), in addition to the appearance of aldosterone induced amiloride sensitive electrogenic $Na^+$ transport.[3] The apical $Na^+$-$H^+$ exchange (NHE-3 isoform) present in distal colonic surface cells is $Cl^-$ independent,[18] and it is perhaps not surprising that in control animals, intracellular pH was similar irrespective of whether surface cells were bathed in $Na_2SO_4$ (6.92 (0.05)) or NaCl (7.07 (0.04)). It is interesting, however, that while EIPA completely reversed the intracellular alkalinisation present in surface cells from $K^+$ loaded animals when bathed in NaCl solution, there was a correspondingly much smaller decrease in intracellular pH when cells were bathed in $Na_2SO_4$ solution, so that the intracellular pH in the presence of EIPA remained above that seen in cells from control animals. This observation is not readily explained, but it is feasible that $Cl^-$ removal decreases the EIPA sensitivity of the classic $Cl^-$ independent $Na^+$-$H^+$ exchange.

Compared with dietary $K^+$ loading, the stimulation of apical $K^+$ channels in surface colonocytes and the enhanced active $K^+$ secretion seen in rat distal colon during dietary $Na^+$ depletion are associated with much higher circulating plasma levels of aldosterone which completely suppress apical $Na^+$-$H^+$ exchange.[13 19 20] If we assume that the changes in distal colonic ion transport occurring in $Na^+$ depleted animals are also accompanied by intracellular alkalinisation, then mechanisms other than enhanced apical $Na^+$-$H^+$ exchange must exist to raise intracellular pH. One possibility is the apical $K^+$,$H^+$-ATPase present in surface colonocytes of the distal colon, as the activity of this $K^+$-$H^+$ exchanger is enhanced by hyperaldosteronism secondary to dietary $Na^+$ depletion.[21] In addition, the basolateral $Na^+$-$H^+$ exchange identified in rat distal colon[22] may be upregulated by aldosterone, as well as sharing EIPA sensitivity with the other NHE isoforms.

Our finding that dietary $K^+$ loading increases intracellular pH in surface cells of rat distal colon fits well with the pH sensitivity of the 210 pS apical $K^+$ channels seen in the inside-out patches. The twofold increase in $K^+$ channel activity seen at hyperpolarising voltages when the bath pH was increased from 7.40 to 7.60 suggests that an increase in intracellular pH of similar magnitude plays an important role in enhancing both apical $K^+$ conductance and net $K^+$ secretion during dietary $K^+$ loading. The ability of EIPA to elicit a fully reversible 50% decrease in $K^+$ channel activity in cell attached patches on surface cells from $K^+$ loaded animals points to a close interaction between enhanced $Na^+$-$H^+$ exchange, intracellular alkalinisation, and the increased levels of 210 pS apical $K^+$ channel activity that occur in this example of colonic $K^+$ adaptation. Studies with conventional and $H^+$ selective microelectrodes have also shown that intracellular alkalinisation secondary to removal of the $Cl^-$-$HCO_3^-$ exchange mechanism is associated with a rise in apical $K^+$ conductance in surface cells of rabbit distal colon.[23]

In summary, we have now identified 210 pS $K^+$ channels in the apical membrane of surface cells in rat distal colon which are regulated by intracellular pH, in addition to intracellular $Ca^{2+}$ and membrane voltage.[9] They probably play a critical role in setting the level of distal colonic $K^+$ secretion. In contrast with distal colon from rats fed a normal diet, human distal colon actively secretes $K^+$ under voltage clamp conditions,[24] and there is evidence from microelectrode and current fluctuation analysis studies for an apical $K^+$ conductance in surface cells in this colonic segment.[25] Interestingly, in preliminary studies we have identified high levels of 220 pS $K^+$ channel activity in the apical membrane of surface cells surrounding the openings of crypts isolated from human distal colonic biopsy samples (G I Sandle and I Butterfield, unpublished data). These provide a focus for studying the mechanisms of enhanced colonic $K^+$ secretion in a variety of diseases.

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