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EFFECTS OF SECRETAGOGUES ON THE K^+ PERMEABILITY OF MUCOSAL AND SEROSAL BORDERS OF RABBIT COLONIC MUCOSA

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(1) K^+ efflux rates from the mucosal and serosal surfaces of sheets of rabbit colonic mucosa have been determined by measuring net K^+ loss into K^+-free Ringer solution bathing each side of the tissue. (2) Initially, there is a high rate of K^+ loss from the tissue, this falls to a lower steady-state rate after 20 min. Loss of K^+ from the tissue into the serosal bath is 6–8-fold faster than loss to the mucosal bath. (3) A number of intestinal secretagogues, e.g. theophylline, cyclic AMP, carbachol, ionophore A23187, as well as the laxative bisacodyl, raise the K^+ efflux rate across the mucosal border by 200–300%. In the case of K^+ efflux induced by carbachol the effect is shown to be dependent on raised levels of intracellular Ca^{2+}. Ca^{2+}-calmodulin complex does not appear to be be involved in activation of K^+ efflux across the mucosal border. (4) Amiloride does not block mucosal K^+ efflux, but tetraethylammonium does inhibit K^+ efflux across the mucosal border, induced by either bisacodyl or raised intracellular Ca^{2+}. (5) The results suggest that laxatives may increase the rate of K^+ secretion into the colonic lumen by raising the K^+ permeability of the mucosal border.

Introduction

The mammalian colon has the capacity to adjust net K^+ transport to varying metabolic requirements [1,2]. There is uncertainty, at present, about the way that the colon regulates this K^+ secretion. Some claim that K^+ secretion is active [3–5] and that laxatives increase this secretion [6,7]. In K^+-deficient animals, net colonic secretion is reduced to zero, although the electrochemical potential gradient of K^+ across the colon favours K^+ accumulation within the lumen [8].

Frizzell et al. [9] observed zero net K^+ flux across rabbit descending colonic mucosa in the short circuit current condition, although they found a high K^+ permeability of the paracellular pathway. The evidence for a high paracellular K^+ conductance has been disputed by Wills et al. [10] who found instead that the baso-lateral membranes have a high conductance towards K^+ but that the paracellular pathway has a low conductance for both Na^+ and K^+.

In this present study, paracellular K^+ movements are eliminated by bathing the colon in low K^+ Ringer solution. This technique has been used to investigate the possible role of cytosolic Ca^{2+} in control of the colon epithelial membrane permeability to K^+.

The findings reported in this paper suggest that K^+ permeability of the mucosal membrane is increased when Ca^{2+} activity is raised and that these changes may explain the observed changes in net transepithelial K^+ secretion when the tissue is exposed to secretagogues.

Materials and Methods

Animals. Male white New Zealand rabbits (2–3 kg) were killed by intravenous injection of Nembutal. The distal colon was rapidly excised, washed, opened longitudinally and stripped of its serosa and muscle

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layers, using the technique described by Frizzell et al. [9] in which the whole mucosa is sheared from the underlying muscle layers with a glass slide.

Flux measurements in flux chambers. The stripped mucosa was mounted in temperature-regulated (37°C) Ussing-type chambers gassed with 5% CO_2/95% O_2. 5 ml solution was added to the mucosal and serosal chambers. 2.5 ml samples were taken every 20 min, for 80–120 min, from both serosal and mucosal compartments and the volume was immediately replaced with 2.5 ml fresh solution. K^+ remaining in the tissue at the end of the efflux period was extracted overnight with 2 ml 0.1 M HNO_3. The K^+ in all samples was analysed by flame photometry using a Corning 400 flame photometer.

K^+ efflux from tissue to the mucosal and serosal chambers was studied in four to seven 20-min consecutive periods. K^+ flux rates were calculated for each period (p) from:

$$K^+ \text{ flux rate}_p = \left(Qm_p \text{ (or } Qs_p) \ (Q_{\text{tissue}} + \sum_{i=p}^{p_1} Qm_i + \sum_{i=p}^{p_1} Qs_i\right) \times 1/t_p$$

where Qm_p or Qs_p are the μmol of K^+ that appear in the mucosal (m) or serosal (s) chamber, during the period (p) considered; Q_{tissue} is the final μmol of K^+ in tissue; $\Sigma_{i=p}^{p_1} Qm_i$ is the μmol of K^+ that appear in mucosal chamber from period p to the last period (p_1); $\Sigma_{i=p}^{p_1} Qs_i$ is the μmol of K^+ that appear in the serosal chamber from period p to the last period (p_1); t_p is the duration of the period, expressed in hours.

Experiments with unmounted tissue. The colonic mucosa was cut into 50–100-mg pieces which were preincubated for 20 min in a beaker containing 25 ml low K^+ Ringer's solution. The bathing solution was maintained at 37°C and gassed with 5% CO_2/95% O_2. After preincubation some tissues were dried on filter paper and weighed, others were transferred to test solutions and incubated for 90 min. Extraction of K^+ from tissues and K^+ determination was as described before. Results were expressed as μmol K^+/g wet weight.

Solutions. Ringer's solution contained 140 mM NaCl, 10 mM $KHCO_3$, 2.4 mM K_2PO_4, 0.4 mM KH_2PO_4 and 1.2 mM $CaCl_2$. Low K^+ Ringer's solution was prepared by replacing the K^+ salts by the suitable Na^+ salts. High Ca/low K^+ Ringer's solution was prepared by raising the $CaCl_2$ concentration to 5 mM.

Materials. Cyclic AMP and tetraethylammonium were obtained from Sigma Chemicals Ltd.; theophylline and carbachol were purchased from B.D.H.; bisacodyl was a gift from Karl Thomae. Experiments with bisacodyl were done using its free diphenol which was prepared by acid (1 M HCl) hydrolysis and then dissolved in ethanol at a concentration of 50 mM (stock solution). Aliquots of the ethanolic solution of the diphenolic derivative of bisacodyl were added to the Ringer's solution to give the appropriate final concentration. Trifluoperazine was a gift from Smith, Kline and French, Welwyn Garden. RMI 12330A was a gift from Merrell, Cincinnati, OH.

Statistical methods. Errors of grouped results are expressed as the standard error of the mean. Statistical comparisons of different treatments on the same animal were made using paired Student's t-tests. In these tests, the mean difference of paired data was calculated from:

$$d_{i_{i,j}} = K^+ \text{ flux rate}_{a_i} - K^+ \text{ flux rate}_{b_j}$$
being $1 < i < n_a$, $1 < j < n_b$)

where a and b refer to the conditions compared and the subscripts i and j to the observation made for each condition.

Results

Characteristics of K^+ efflux from stripped colonic mucosa

Pieces of isolated colonic mucosa were mounted in the chambers and bathed with Ringer's solution in which potassium salts were replaced by the corresponding sodium salts (low K^+ Ringer's solution). With this preparation there is net potassium loss from the tissue and both mucosal and serosal K^+ efflux rates can be determined. The efflux of K^+ was studied for 100 min and the rates are shown in Fig. 1. The following points can be obtained from this figure: firstly, K^+ efflux across the serosal border was 6–8 times faster than across the mucosal border; secondly, initial rates were always significantly higher than those obtained in the successive 20-min periods; thirdly, preincubation of the tissue in low K^+ Ringer's solution for 20 min resulted in a significant reduction

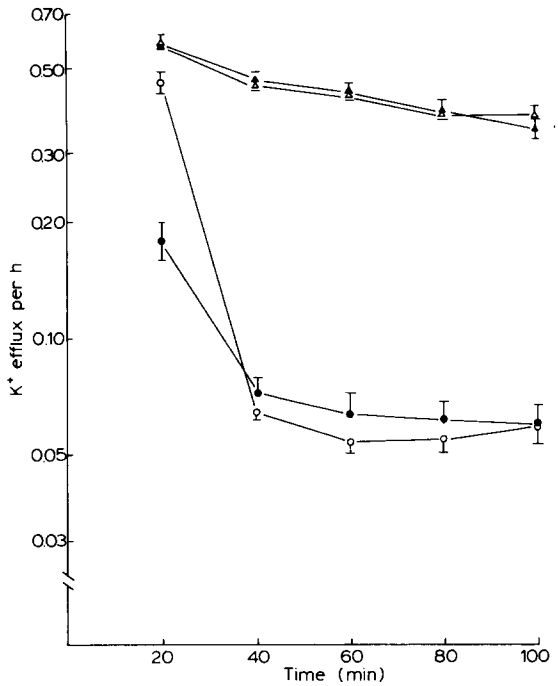

Fig. 1. K^+ efflux rates from sheets of isolated colon mucosa as a function of time. Circles indicate mucosal rates with (•) or without (○) a 20-min preincubation period in low K^+ Ringer's solution. Triangles indicate serosal rates with (▲) or without (△) preincubation. Each point is the mean ± S.E. of 13–30 separate determinations. Preincubation only affected initial (20 min) mucosal rate.

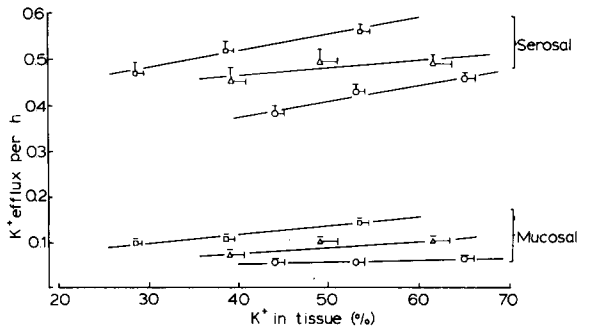

Fig. 2. Relation between mucosal and serosal efflux rates and the percentage of K^+ remaining in tissue. Rate constants plotted correspond to 2nd, 3rd and 4th periods (40, 60 and 80 min from the beginning of the experiment, respectively). Circles, control (42–43); triangles, theophylline (10 mM) present in both mucosal and serosal solutions (20–22); squares, bisacodyl (0.01 mM) present in the bathing solutions (9–12). Numbers in parenthesis refer to number of experiments. Values are presented as mean ± S.E.

in the initial K^+ mucosal rate and was without effect on subsequent or serosal K^+ efflux constants.

Fig. 2. shows K^+ efflux rates calculated at 40, 60 and 80 min plotted against the percentage of initial K^+ remaining in tissue. The figure shows that K^+ efflux had little or no dependence on K^+ content of the tissue. The same result was also obtained in the presence of either theophylline or bisacodyl. The high rate of K^+ loss across the serosal membrane is similar to that reported by Wills et al. [10].

Effects of theophylline and ouabain on K^+ efflux

Table I shows that theophylline, a small intestinal secretagogue [11], when added to both the mucosal and serosal bathing solutions increases K^+ efflux during the period, 40–80 min after exposure to the drug. The initial 40 min is excluded from this analysis to avoid the fast, possibly extracellular component, of K^+ efflux. The percentage increase in mucosal exit in the presence of theophylline is 54% and the serosal K^+ efflux is raised by 13%.

Ouabain (0.1 mM) increases K^+ loss across the serosal border to a much larger extent (90%) than theophylline. This increased serosal K^+ loss is attributable to inhibition of the $(Na^+ + K^+)$-ATPase at the baso-lateral border of the tissue which normally recaptures K^+ lost to the submucosal layers. It will be noted that with ouabain present there is also a significant increase in K^+ loss across the mucosal border (71%). Because theophylline and ouabain have additive effects on K^+ loss across the mucosal border ($P < 0.05$), we deduce that the theophylline-dependent increase in K^+ loss is due to permeability changes independent of effects in the Na-pump activity. To corroborate and test this view, we observed the effect of theophylline on net K^+ loss from pieces of colonic mucosa incubated in Ringer's solution with K^+ concentrations varying from 0 to 25 mM (see Materials and Methods), to ascertain if theophylline affected K^+ loss from tissue with fully active Na-pump function. (Table IB). At all concentrations of K^+/Ringer's solution, significantly less K^+ remained within the tissue exposed to theophylline than controls. When K^+/Ringer's solution is raised to 5 mM, no significant

TABLE IA

NET K^+ EFFLUX FROM SHEETS OF ISOLATED RABBIT COLON MUCOSA

The mucosal and serosal bathing solutions are identical. All values are expressed as mean ± S.E. Test vs. control: [a] $P < 0.001$; [b] $P < 0.05$; [c] $P < 0.001$, different from ouabain.

Condition	K^+ efflux rate per h			
	Mucosal	*n*	Serosal	*n*
Control	0.061 ± 0.002	128	0.425 ± 0.006	126
Theophylline (10 mM)	0.094 ± 0.005 [a]	65	0.479 ± 0.012 [a]	63
Ouabain (0.1 mM)	0.106 ± 0.009 [a]	13	0.805 ± 0.066 [a]	13
Ouabain + theophylline	0.130 ± 0.008 [b]	15	0.586 ± 0.051 [c]	14
Cyclic AMP (5 mM)	0.085 ± 0.013 [a]	18	0.398 ± 0.020	18

net K^+ loss from controls after the 90 min incubation period was observed: K^+/Ringer's solution had to be raised to 15 mM before K^+ loss was prevented from tissue exposed to theophylline. These results confirm that theophylline accelerates K^+ loss from rabbit colon and indicate that the K^+ permeability increase is independent of changes in Na-pump activity.

TABLE IB

FINAL K^+ CONCENTRATION IN PIECES OF COLONIC MUCOSA AFTER 90 MIN INCUBATION IN VARYING K^+ CONCENTRATIONS; EFFECT OF THEOPHYLLINE (10 mM)

Condition	*n*	Final K^+ concn. (μmol/g wt wt.) mean ± S.E.	Significance (*P*)
Control (K^+ 0 mM)	40	46.28 ± 0.97	<0.001
Theophylline	24	38.88 ± 1.31	
Control (K^+ 1 mM)	4	42.91 ± 4.85	<0.001
Theophylline	5	25.70 ± 1.47	
Control (K^+ 2 mM)	3	41.83 ± 3.38	<0.05
Theophylline	5	32.37 ± 2.68	
Control (K^+ 5 mM)	8	51.39 ± 2.31	<0.001
Theophylline	9	38.82 ± 1.88	
Control (K^+ 10 mM)	3	57.54 ± 2.96	<0.05
Theophylline	3	48.24 ± 1.35	
Control (K^+ 15 mM)	9	68.37 ± 1.70	<0.05
Theophylline	8	56.40 ± 2.84	
Control (K^+ 25 mM)	9	81.37 ± 3.95	<0.001
Theophylline	8	59.41 ± 3.50	

Effect of carbachol and A23187 on K^+ efflux

Since theophylline raises cytoplasmic free Ca^{2+} in rabbit ileal mucosa [12] and in rabbit colonic mucosa (Ilundain, A., and Naftalin, R.J., unpublished data), we considered that K^+ efflux might be related to intracellular free Ca^{2+}. To test this hypothesis, we observed the effect of carbachol on the rate of K^+ loss. Carbachol, a muscarinic agonist, opens Ca^{2+} channels and thereby increases the K^+ permeability of the membranes of some secretory epithelia [13]. Also, carbachol is without any effect on cyclic nucleotide levels within epithelia [14,15]. When Ca^{2+} in Ringer's solution is at its normal concentration (Ca^{2+} = 1.2 mM), carbachol (0.5 mM) has no significant effect on K^+ efflux across the mucosal border. However, when Ca^{2+} in Ringer's solution is raised to 5 mM, a significant stimulation of mucosal K^+ efflux is observed ($P < 0.001$). When Ca^{2+} in Ringer's solution is reduced to 0.1 mM in the presence of carbachol, K^+ efflux across the mucosal border falls below that observed with Ca^{2+} at 1.2 mM in Ringer's solution ($P < 0.01$). (Table II and Fig. 3). K^+ loss across the serosal border is significantly reduced by carbachol (Table II), however, there is no obvious relationship between K^+ efflux across the serosal border and Ca^{2+} and in Ringer's solution (Fig. 3).

When the calcium ionophore, A23187, (Calbiochem), is added to both the mucosal and serosal bathing solutions (Ca^{2+} 1.2 mM), there is a small, but significant increase ($P < 0.05$) in K^+ loss across the mucosal border, K^+ loss across the serosal border is unaffected by the ionophore (Table II).

These results indicate that increased entry of Ca^{2+}

TABLE II

EFFECTS OF AGENTS WHICH RAISE INTRACELLULAR Ca^{2+}

Condition	*n*	K^+ efflux rate per h	
		Mucosal	Serosal
Control	128	0.061 ± 0.002	0.425 ± 0.006
Carbachol (0.5 mM); Ca^{2+} = 0.1 mM	12	0.046 ± 0.004 [a]	0.353 ± 0.016 [a]
Carbachol (0.5 mM); Ca^{2+} = 1.2 mM	21	0.066 ± 0.008	0.326 ± 0.01 [b]
Carbachol (0.5 mM); Ca^{2+} = 5 mM	56	0.092 ± 0.005 [b]	0.426 ± 0.009
Cyclic AMP (5 mM); Ca^{2+} = 5 mM	9	0.098 ± 0.010	0.456 ± 0.019
Carbachol + cyclic AMP; Ca^{2+} = 5 mM	9	0.128 ± 0.015 [c,b]	0.432 ± 0.023
A23187; Ca^{2+} = 1.2 mM	9	0.077 ± 0.009 [a]	0.479 ± 0.032

Test vs. control: [a] $P < 0.01$, [b] $P < 0.001$; test vs. carbachol (Ca^{2+} = 5 mM): [c] $P < 0.05$.

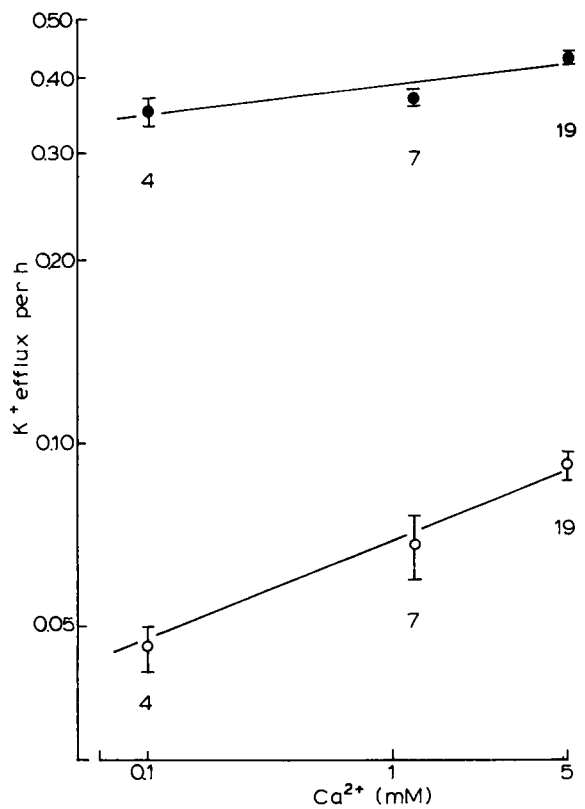

Fig. 3. Effect of varying Ca^{2+} concentration on carbachol-induced K^+ secretion. Mucosal (○) and serosal (●) K^+ efflux rates are shown. Carbachol (0.5 mM) was present in both solutions. Values are presented as mean ± S.E. The figures below the points show the number of experiments.

stimulates K^+ exit across the mucosal border, but has no marked effect on serosal K^+ exit. This differs from the effects of theophylline on K^+ release. With theophylline both mucosal and serosal border K^+ permeability are increased. This difference could result from compartmentalization of intracellular Ca^{2+} or the greater efficacy of theophylline in raising intracellular Ca^{2+}, or more probably from the different effects of carbachol and theophylline on membrane potential (see Discussion).

Effect of cyclic AMP

Cyclic AMP, when added to the bathing solution, has been shown to induce Cl^- secretion by rabbit colonic mucosa [15] and to release Ca^{2+} stored within the cells into the cytosol. Cyclic AMP when added to the bathing solution (Table II) also increases K^+ efflux across the mucosal border. No effect on serosal K^+ loss is observed.

When cyclic AMP (5 mM) is added to Ringer's solution (Ca^{2+} = 5 mM) containing carbachol (0.5 mM) there is an additional increase in mucosal permeability above that seen with cyclic AMP. This increase may be due to the additive effect of these agents in increasing intracellular Ca^{2+} from both intracellular and extracellular sources.

Effects of trifluoperazine (stelazine) and RMI 12330A

Trifluoperazine and RMI 12330A have been used previously to prevent secretion by rabbit ileum induced by agents which raise intracellular Ca^{2+}, by

activating release from stored intracellular sources [12]. They act by preventing Ca^{2+}-calmodulin complex from activating brush-border Cl^- permeability. Table III shows that neither drug prevents theophylline from stimulating K^+ permeability of the mucosal border of rabbit colon. At higher concentrations (0.1 mM) both drugs increase K^+ permeability. From these results we deduce that calmodulin is not a necessary intermediate in the process which activates K^+ permeability of the apical border of rabbit colon.

Effects of bisacodyl

Bisacodyl (Karl Thomae) is a diphenolic laxative, closely similar in both structure and action to phenolphthalein, it is known to promote Na^+ and K^+ secretion into both large and small intestine [6]. The concentration dependence of bisacodyl-induced K^+ release from rabbit colon was first investigated by us using pieces of colonic mucosa. Above 10^{-6} M, bisacodyl produced a significant increase in K^+ release. The experiments on K^+ release from colonic mucosal sheets mounted in Ussing-type chambers used bisacodyl at 10^{-5} M. This concentration was gauged to give a maximal stimulation of K^+ efflux.

It can be seen from Table IV that when bisacodyl was present in both mucosal and serosal solutions, K^+ efflux across both the mucosal and serosal borders is stimulated.

Tetraethylammonium is a ganglionic blocking agent, known to reduce K^+ conductance across nerve and muscle membranes [16,17]. Tetraethylammonium is an effective inhibitor of the bisacodyl-dependent increase in K^+ permeability of the mucosal border (Fig. 4). However, it is a completely ineffective blocker of the K^+ permeability of the serosal border, nor does tetraethylammonium block K^+ release from the mucosal side of unstimulated tissue. Tetraethylammonium is also an effective inhibitor of carbachol-stimulated K^+ release across the mucosal border (Table IV). This latter finding indicates that the K^+ channels induced in the mucosal border by raised intracellular Ca^{2+} have similar properties to those induced by bisacodyl.

Effect of amiloride

Amiloride is a diuretic which acts by inhibiting Na^+ movement across the apical membranes of tight epithelia. Amiloride blocks Na^+ entry, net transepithelial Na^+ transport, reduced the potential difference and short-circuit current to zero and hyperpolarizes the apical and basal cell membranes of rabbit descending colon [9,18,19]. Yorio and Bentley [5] demonstrated net K^+ secretion across rabbit colon (in vitro) in the short-circuit current condition and showed that amiloride is without effect on this K^+ secretion.

The results in Table IV shows that amiloride has no effect on K^+ exit across the mucosal border of either control tissue or tissue exposed to bisacodyl, where K^+ efflux is raised. However, K^+ exit across the serosal border is reduced in the presence of amiloride. This reduced K^+ leakage may be due to the hyperpolarization of the baso-lateral membranes following exposure to the drug [19]. These results are consistent with those of Yorio and Bentley [5], since they indicate that K^+ secretion across the mucosal

TABLE III

EFFECT OF TRIFLUOPERAZINE AND RMI 12330A ON K^+ LOSS FROM RABBIT COLONIC MUCOSA

K^+ efflux rate from mucosal and serosal sides of isolated rabbit colon. Test vs. control (same column): [a] $P < 0.05$; [b] $P < 0.001$.

Drug	Concn. (μM)	K^+ efflux rate per h					
		Control			Theophylline (10 mM)		
		n	Mucosal	Serosal	*n*	Mucosal	Serosal
Trifluoperazine	0	128	0.061 ± 0.002	0.425 ± 0.0005	65	0.094 ± 0.005 [b]	0.479 ± 0.012
	5	9	0.042 ± 0.005 [a]	0.363 ± 0.014 [a]	9	0.077 ± 0.008	0.391 ± 0.019
	100	17	0.158 ± 0.018 [b]	0.430 ± 0.011	14	0.237 ± 0.015 [b]	0.472 ± 0.028
RMI 12330A	100	9	0.161 ± 0.02 [b]	0.695 ± 0.026 [b]	9	0.215 ± 0.022 [b]	0.617 ± 0.037 [b]

TABLE IV

NET K^+ EFFLUX FROM SHEETS OF ISOLATED RABBIT COLONIC MUCOSA

The mucosal and serosal bathing solutions are identical. All errors are S.E. Test vs. control: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$; test vs. bisacodyl: [d] $P < 0.001$; test vs. carbachol (Ca^{2+} = 5 mM): [e] $P < 0.01$.

Condition	n	K^+ efflux rate per h	
		Mucosal	Serosal
Control	128	0.061 ± 0.002	0.425 ± 0.006
Bisacodyl (10^{-5} M)	36	0.118 ± 0.005 [c]	0.516 ± 0.013 [c]
Tetraethylammonium (5 mM)	9	0.028 ± 0.002 [b]	0.493 ± 0.008 [a]
Bisacodyl + tetraethylammonium	18	0.077 ± 0.004 [d]	0.584 ± 0.014 [c]
Amiloride (0.1 mM)	9	0.058 ± 0.007	0.290 ± 0.006 [c]
Bisacodyl + amiloride	9	0.128 ± 0.017 [c]	0.407 ± 0.019 [d]
Carbachol (0.5 mM)	56	0.092 ± 0.005 [c]	0.426 ± 0.009
Carbachol (0.5 mM)(Ca^{2+} = 5 mM) + tetraethylammonium (5 mM)	9	0.056 ± 0.004 [e]	0.328 ± 0.011 [b]

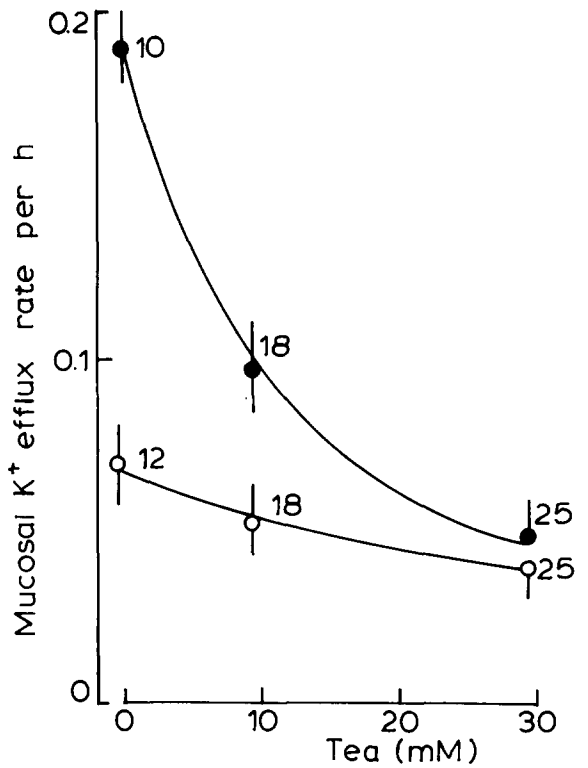

Fig. 4. Effect of varying concentrations of tetraethylammonium (TEA) on the mucosal bisacodyl-induced K^+ secretion. Bisacodyl (0.02 mM) and/or tetraethylammonium were present in both mucosal and serosal solutions. ○, tetraethylammonium; ●, tetraethylammonium plus bisacodyl. Values are presented as mean ± S.E. The figures below the points show the number of experiments.

membrane is unaffected by changes in the electrical potential gradient. The results also show that K^+ loss across the mucosal membrane is independent of the net activity of the Na^+ pump.

Discussion

As we noted in the Introduction, several conflicting views have been expressed concerning the cause of the high lumenal K^+ concentration present in colon. Bastl et al. [8] have concluded that K^+ is not secreted actively by rat colon, but merely equilibrates across the mucosa according to its electrochemical potential. Frizzell et al. [9] found no net K^+ flux across rabbit colonic mucosa in the short-circuit condition, although they did record a large voltage-dependent K^+ flow across the tissue which they defined as paracellular flow. The paracellular permeability ratios of K^+ : Na^+ : Cl^- were found to be 1 : 0.07 : 0.1. On the other hand, Bentley and Smith [20] have found net K^+ secretion into the lumen of new-born pig colon incubated in the short circuit current condition and Yorio and Bentley [5] have found that K^+ secretion exists across sacs of rabbit descending colon incubated in the short-circuit condition. Amiloride, which entirely blocks active Na^+ absorption across the colon, as well as the transepithelial potential difference and short-circuit current, is without any effect on net K^+ secretion. This suggests that the K^+ secretion is not electrogenic.

Edmunds and Smith [21] observed that the steady-state K^+ concentration within the lumenal contents of rat colon is 50 mM, this is much higher than the values predicted from the K^+ equilibrium potential between blood and lumen. They also found that K^+ transit across rat colon has a rapid transcellular component. This confirms earlier work on K^+ content of human colon [22] Frizzell and Jennings [23] noted that K^+ uptake across the serosal border of rabbit colon is very rapid, accounting for 90% of the total K^+ influx into the tissue. An electrophysiological study on rabbit colon by Wills et al. [10] confirmed that K^+ conductance of the basolateral border is high, 15 times higher than Na^+ and 10-fold higher than Cl^-, but they did not confirm that the paracellular shunt has any K^+ selectivity over Na^+. The apical border constitutes the major barrier to K^+ movement across the colon.

These latter findings are confirmed by the results of this paper. The K^+ permeability of the serosal border, as determined by net efflux, is very close to that found by Wills et al. [10]. It is also well established that both in diarrhoea and with laxative action there is an increased net K^+ secretion into the colonic lumen in vivo [6,7].

Several theories have been advanced as general explanations of how laxatives induce colonic secretion. Increased paracellular permeability to both large and small molecules [24]; prostaglandins release activating adenylate cyclase which in turn raises intracellular cyclic AMP, which activates secretion [25,26]; reduction in $(Na^+ + K^+)$-ATPase activity [27] have all been proposed. Powell et al. [28] have shown that the diphenolic laxative drug, phenolphthalein, when applied to the mucosal side of rabbit ileum induces electrolyte secretion, but when it is applied to the serosal side it enhances absorption. Phenolphthalein is without any effect on tissue cyclic nucleotide metabolism or Na-pump activity, thus views that the observed metabolic changes are specifically related to either absorption or secretion will have to be revised.

Frizzell [15] showed that raising intracellular Ca^{2+} with the Ca^{2+} ionophore A23187 increases Cl^- conductance across the apical border of rabbit colon, and also suggested that raised cyclic AMP raises free intracellular Ca^{2+} by releasing bound Ca^{2+} into the cytosol. Raised cytosolic Ca^{2+} has been shown to increase K^+ conductance across the membrane of a number of different tissues, particularly salivary gland [13] and red cells [29,30].

The role of Ca^{2+} in colonic secretion

Raising intracellular Ca^{2+} concentration within colonic epithelial cells results in an increased rate of K^+ loss across the mucosal border. However, although raised intracellular Ca^{2+} is a determinant of mucosal border K^+ permeability, it is apparent that other factors may also be involved in activation of K^+ permeability. The results shown in Table II indicate that cyclic AMP has an additive effect to that carbachol + Ca^{2+} (5 mM) in increasing K^+ permeability of the mucosal border. Additional evidence in favour of the view that both cyclic AMP and Ca^{2+} may have a role in modulation of mucosal border K^+ permeability is the relative inefficacy of A23187 or carbachol in increasing mucosal border K^+ permeability: both these treatments increase intracellular Ca^{2+} without affecting cyclic AMP [12,14]; whereas theophylline, which increases both intracellular Ca^{2+} and cyclic AMP [12], has a larger effect on the K^+ permeability of the mucosal border than cyclic AMP alone or carbachol with Ca^{2+} in Ringer's solution = 1.2 mM alone (Tables I and II).

Calcium-dependent activation of mucosal border K^+ permeability is unlikely to be mediated by calmodulin, since drugs which inhibit calmodulin-activated Cl^- permeability in rabbit ileum (trifluoperazine and RMI 12330A [12,31]) do not inhibit the theophylline-dependent increase in K^+ permeability (Table III). At high concentrations both these drugs increase the K^+ permeability of the mucosal border. There is no direct evidence suggesting that the drug-induced increase in K^+ permeability depends on either intracellular Ca^{2+} or cyclic AMP.

Likewise, it is uncertain whether or not the action of the laxative bisacodyl on mucosal border K^+ permeability depends on raised intracellular Ca^{2+}. Bisacodyl raises intracellular cyclic AMP by increasing adenylated cyclase activity [27], and hence it is possible that intracellular Ca^{2+} is increased following exposure to the drug [15]. However, raising intracellular cyclic AMP by addition of exogenous cyclic AMP has a much smaller effect on the mucosal K^+ permeability than either high concentrations of bisacodyl, trifluoperazine or RMI 12330A (Tables II

and III). Thus, these drugs may increase the mucosal border K^+ permeability by some mechanism other than by involvement of Ca^{2+} or cyclic AMP.

Although we are uncertain about the precise role of Ca^{2+} in the Ca^{2+}-dependent activation of K^+ permeability by carbachol, it is clear that once the mucosal border K^+ permeability is activated, it can then be inhibited by tetraethylammonium (Table IV and Fig. 4). This indicates that the mucosal K^+ channels are uniform.

There are distinct differences between the properties of the K^+ channels within the mucosal and serosal membranes, The mucosal border K^+ permeability is activated by Ca^{2+}, is insensitive to changes in membrane potential, and binds tetraethylammonium: the serosal K^+ channel is not apparently activated by Ca^{2+}, is not affected by tetraethylammonium and is sensitive to changes in membrane potential. Additionally, K^+ loss across the serosal border is more sensitive to ouabain than is K^+ loss across the mucosal border; however, this difference reflects the location of the $(Na^+ + K^+)$-ATPase rather than any passive permeability property. The increased K^+ loss across the mucosal border observed with ouabain present seems to be due to increased leakage via the cation-selective tight-junction [8] following net K^+ leakage across the baso-lateral membrane into the lateral intercellular spaces, rather than to direct leakage across the apical membranes following depolarization. We infer that this is because mucosal K^+ loss does not respond to the hyperpolarizing changes in membrane potential induced by amiloride (Table IV). Further experiments are required to give a more definitive explanation of the ouabain-dependent increase in K^+ loss.

Laxative-induced increase in K^+ secretion by colon

The increased net K^+ secretion [3,6,21,24] which occurs after exposure to laxatives and the results shown here are consistent with the view that net K^+ secretion arises from increased transcellular K^+ movement, which is due to increased K^+ permeability of the mucosal border. Net K^+ loss does not occur across the serosal border because K^+ is recycled between the cells and lamina propria by $(Na^+ + K^+)$-pump activity counteracting the high rate of K^+ leakage.

Although enhanced net K^+ secretion is readily explained by increased passive K^+ permeability across the mucosal membrane, the high equilibrium concentration of K^+ attained within the lumen of the distal colon (50–90 mM) [4,14] cannot be simply ascribed to an increased rate of passive equilibration of K^+ down its electrochemical potential gradient. The K^+ concentration within the lumen is approximately 5–8 times higher than that predicted from the electrochemical potential for K^+ across either the apical membrane (intracellular K^+ activity 70–90 mM, potential difference across the apical border = –49 ± 5 mV, [19]), or across the whole tissue (transepithelial potential difference 15 ± 5 mV serosa positive, plasma K^+ = 5 mM, [4]).

One cause of the laxative-induced increase in K^+ secretion which has been considered previously is that K^+ release into the colonic lumen is associated with mucous release from goblet cells [32]. We consider that enhanced mucous release may be an important factor in the initial high rate of K^+ release from the tissue. But the sustained increased rate of K^+ release cannot come from goblet cells alone, since the total K^+ which is released during this prolonged period comprises more than 50% of the total tissue K^+ (Figs. 1 and 2).

A possible mode of attaining the high lumenal $[K^+]$ in colon, is that K^+ equilibrates across the mucosal border by an electroneutral process, in which K^+ movement is coupled to passive leakage of Cl^- or HCO_3^- from the tissue. The basic requirements for this coupled process are the coexistence of a high K^+ conductance and a high anion conductance across the mucosal border. The driving force of K^+ accumulation within the lumen would be the albebraic sum of the chemical potential gradients of K^+, Cl^- and HCO_3^- across the mucosal membrane. The coupled electroneutral movement of KCl is unrelated to the electrical gradients either across the apical membrane or across the whole epithelium. In this paper we have shown that raised intracellular Ca^{2+} increases K^+ conductance and previously it has been shown that secretagogues increase Cl^- conductance across the apical border of colon [15], as well as the ileum [12]. The colon also secretes bicarbonate copiously [33]. Thus, the necessary preconditions for electroneutral K^+ transport across the mucosal membrane exist in the secreting colon. Further work is required to test whether electroneutral K^+ flux is present and how it occurs.

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