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Regulation of apical and basolateral K^+ conductances in the rat colon

G. Schultheiß & [1]M. Diener

Institut für Veterinär-Physiologie, Justus-Liebig-Universität Gießen, D-35392 Gießen, Germany

1 Apical administration of an ionophore, nystatin, and basolateral depolarization by K^+ were used to investigate the regulation of apical and basolateral electrogenic transport pathways for K^+ in the rat proximal and distal colon.

2 Administration of nystatin (100 μg ml^{-1} at the mucosal side), in the presence of Na^+ and in the presence of a serosally directed K^+ gradient, stimulate a large increase in short-circuit current (I_{SC}) and tissue conductance in both colonic segments. This response was composed of a pump current generated by the Na^+-K^+-ATPase and of a current across a quinine-sensitive basolateral K^+ conductance.

3 The pump current, measured as Na^+-dependent or scilliroside-sensitive current in the absence of a K^+ gradient, was significantly greater in the distal than in the proximal colon. The pump current was unaltered by pretreatment of the tissue with forskolin (5×10^{-6} mol l^{-1}).

4 The current across the basolateral K^+ conductance, measured as current in the presence of a serosally directed K^+ gradient either in the absence of Na^+ or in the presence of scilliroside, was increased by the cholinoreceptor agonist, carbachol (5×10^{-5} mol l^{-1}), but inhibited by forskolin (5×10^{-6} mol l^{-1}).

5 Basolateral K^+ depolarization induced a negative I_{SC} in both colonic segments, which was inhibited by the K^+ channel blocker quinine (10^{-3} mol l^{-1} at the mucosal side), but was resistant to tetraethylammonium (5×10^{-3} mol l^{-1} at the mucosal side). This K^+ current across an apical K^+ conductance was stimulated in both colonic segments by carbachol, whereas forskolin had no effect, although control experiments revealed that forskolin was still able to open an apical Cl^- conductance under these conditions.

6 These results demonstrate that an increase in intracellular Ca^{2+} concentration induced by carbachol causes an increase in the basolateral and the apical K^+ conductance, thereby inducing K^+ secretion in parallel with an indirect support for Cl^- secretion due to the hyperpolarization of the cell membrane. In contrast, the dominating effect of an increase in the intracellular cyclic AMP concentration is inhibition of a basolateral K^+ conductance; a mechanism which might contribute to the inhibition of K^+ absorption.

Keywords: Ca^{2+}; cyclic AMP; electrolyte transport; K^+ channels; K^+ transport; rat colon

Introduction

The colon plays an important role in K^+ homeostasis, because in contrast to other intestinal segments the colonic epithelium transports this cation not only by paracellular but also by transcellular pathways. The current model of K^+ absorption by the colon (for review see Binder & Sandle, 1994) assumes that K^+ ions are absorbed from the colonic lumen by an H^+-K^+-ATPase, whereas the basolateral exit of absorbed K^+ is mediated by basolateral K^+ channels. Potassium secretion is driven by the intracellular accumulation of K^+ ions which enter the enterocyte by the basolateral Na^+-K^+-Cl^--cotransporter and the Na^+-K^+-ATPase. K^+ leaves the cell via apical K^+ channels (for review see Binder & Sandle, 1994). In addition, there is a passive flux of K^+ across the epithelium across the paracellular pathway (McCabe *et al*., 1986).

Potassium transport is regulated by intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and Ca^{2+}. An increase in the intracellular cyclic AMP concentration induces K^+ secretion and inhibits K^+ absorption (Foster *et al*., 1983). A K^+ secretion is also evoked after an increase in the intracellular Ca^{2+} concentration (McCabe & Smith, 1985). Further control occurs via plasma aldosterone, because a secondary hyperaldosteronism induced by a Na^+-deficient diet is known to stimulate colonic K^+ secretion (Sweiry & Binder, 1989).

The mechanisms underlying this regulation are poorly understood. Recently, it has been shown by efflux experiments that an increase in intracellular cyclic AMP concentration induced by the activator of the adenylate cyclase, forskolin, causes a redistribution of cellular K^+ permeability from a predominant basolateral one to a near equal apical and basolateral K^+ permeability. The reason for this redistribution seems to be a decrease in basolateral K^+ conductance as indicated by whole-cell patch-clamp experiments (Diener *et al*., 1996). Taken together with the depolarization induced by the opening of apical Cl^- channels, this redistribution of the cellular K^+ conductance might be responsible for an increased efflux of K^+ across apical K^+ channels. However, the question of whether the stimulation of K^+ secretion by cyclic AMP is only caused by the increase in driving force for K^+ secretion, i.e. the cellular depolarization, or whether cyclic AMP in addition opens an apical K^+ conductance, could not be resolved, because in whole-cell patch-clamp experiments only total K^+ conductance can be measured. Therefore, in the present experiments, conductive K^+ transport across the apical and the basolateral membrane was studied in the intact colonic epithelium by the use of the ionophore, nystatin, in order to bypass the apical membrane, and by basolateral K^+ depolarization in order to bypass the basolateral membrane. The regulation of K^+ currents across both membranes by forskolin and carbachol, i.e. agonists acting at the intracellular cyclic AMP and the intracellular Ca^{2+} signalling pathway, respectively, was investigated.

[1] Author for correspondence:

Methods

Solutions

The standard buffer for the Ussing chamber experiments was Parson's solution (Parsons & Paterson, 1965) containing (mmol l^{-1}): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12 gassed with carbogen (5% CO_2 in 95% O_2); pH was 7.4. For the Na^+-free solution, NaCl was replaced by N-methyl-D-glucamine ($NMDG^+$) chloride. In order to apply a K^+ gradient, the KCl concentration in this buffer was increased to 13.5 mmol l^{-1} while the NaCl concentration was reduced in order to maintain isoosmolarity. For the depolarization of the basolateral membrane, a 111.5 mmol l^{-1} KCl solution was used, in which NaCl was equimolarly replaced by KCl. In the Cl^--free buffers, NaCl and KCl was substituted by Na gluconate (NaGluc) and K gluconate (KGluc), respectively.

The HCO_3^--free Tyrode solution had the following composition (mmol l^{-1}): NaCl 140, KCl 5.4, $CaCl_2$ 1.25, $MgSO_4$ 1, HEPES (N-(2-hydroxyethyl)piperazine-N′-2-ethanesulphonic acid) 10 and glucose 12.2. To obtain a Cl^--free Tyrode solution, NaCl and KCl were substituted by NaGluc and KGluc, respectively. For the K^+ depolarization under Cl^-- and HCO_3^--free conditions, NaCl and KCl were both substituted by KGluc. All HCO_3^--free solutions were gassed with O_2; pH was 7.4.

Tissue preparation

Female Wistar rats were used weighing 180–240 g. The animals had free access to water and a standard rat diet (diet no. C1000, Altromin, Lange, Germany) until the day of the experiment. In one experimental series, the animals were kept for two weeks on a NaCl-poor diet (diet no. C1036, Altromin, Lange, Germany). Animals were killed by a blow on the head followed by exsanguination. The serosa and muscularis propria were stripped away to obtain a mucosa-submucosa preparation of the colon. Briefly, the colon was placed on a small plastic rod with a diameter of 5 mm. A circular incision was made near the anal end with a blunt scalpel and the serosa together with the lamina propria were gently removed in a proximal direction (Andres *et al*., 1985). The appearance of palm-like striae was used to define the beginning of the proximal colon (Lindström *et al*., 1979).

Short-circuit current measurement

The mucosa-submucosa preparation was fixed in a modified Ussing chamber, bathed with a volume of 4 ml on each side of the mucosa (Andres *et al*., 1985). The tissue was incubated at 37°C in Parsons solution and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen, Germany) with correction for solution resistance. Tissue conductance (G_t) was measured every min by the voltage deviation induced by a current pulse (± 50 μA, duration 200 ms) under open-circuit conditions. Short-circuit current (I_{SC}) was continuously recorded on a chart-recorder. In the figures, I_{SC} and G_t data were averaged every min. For fast responses, i.e. the I_{SC} induced by carbachol, current data were printed every 6 s and averaged at this time interval for the respective figures. In the tables, the maximal increase in I_{SC} (ΔI_{SC}) induced by a drug or K^+ depolarization is given.

Measurement of apical and basolateral K^+ currents

The apical membrane was permeabilized by apical administration of nystatin (100 μg/ml^{-1}) dissolved in dimethylsulphoxide (DMSO; final concentration 0.2% v/v). Nystatin was ultrasonicated immediately before use. The I_{SC} response to the ionophore was tested in the presence and absence of a K^+ gradient (13.5 mmol l^{-1} at the mucosal and 4.5 mmol l^{-1} at the serosal side). In order to depolarize the basolateral membrane, the tissue was exposed to a high K^+ buffer (111.5 mmol l^{-1} KCl) at the serosal side.

Drugs

Bumetanide, forskolin, glibenclamide (generous gift from Boehringer Mannheim, Mannheim, Germany), indomethacin and quinine were dissolved in ethanol (final maximal concentration 0.25%, v/v). Scilliroside (generous gift from Sandoz, Basel, Switzerland) was dissolved in methanol (final concentration 0.25%, v/v). All other drugs were dissolved in aqueous stock solutions diluted in salt buffer just before use. Tetraethylammonium (TEA) was added as the chloride salt. If not indicated differently, drugs were from Sigma, Deisenhofen, Germany.

Statistics

Values are presented as means ± s.e.mean. When the means of several groups had to be compared, first analysis of variances was performed. If the analysis of variances indicated significant differences between the groups investigated, further comparison was carried out by Student's *t* test or by the U-test. An *F*-test decided which test method was to be used. Both paired and unpaired two-tailed Student's *t* tests were applied as indicated. $P<0.05$ was considered to be statistically significant.

Results

Effect of nystatin in the presence of a K^+ gradient and in the presence of Na^+ ions

Basal I_{SC} in the rat distal colon bathed with the standard Parsons solution amounted to 2.6 ± 0.4 μEq h^{-1} cm^{-2} at a G_t of 9.4 ± 1.1 mS cm^{-2} ($n=17$). The corresponding values for

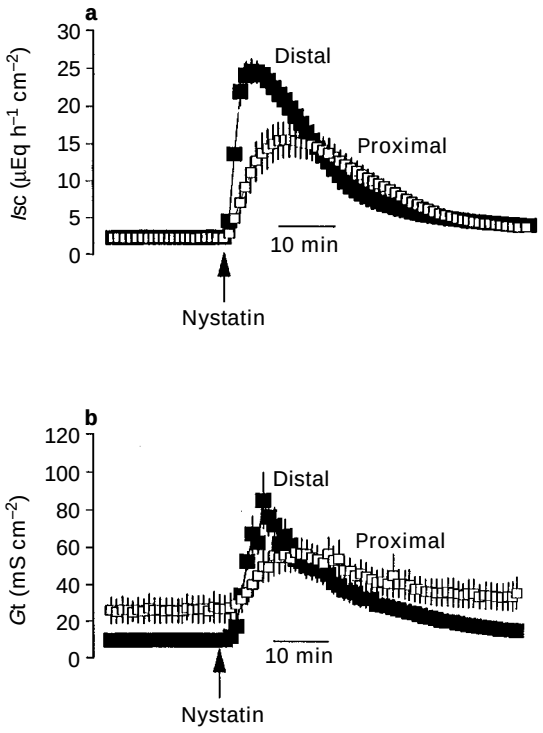

Figure 1 Effect of nystatin (100 μg ml^{-1} at the mucosal side) on I_{SC} (a) and G_t (b) in the distal and proximal rat colon. The experiments were performed in the presence of Na^+ and in the presence of a K^+ gradient (13.5 mmol l^{-1} at the mucosal and 4.5 mmol l^{-1} at the serosal side). Values are means (symbols) and s.e.mean (vertical lines), $n=11-12$.

Table 1 Effect of K^+ channel blockers on total nystatin response

	Distal colon		*Proximal colon*		
	ΔI_{sc} (μEq h^{-1} cm^{-2})	ΔG_t (mS cm^{-2})	ΔI_{sc} (μEq h^{-1} cm^{-2})	ΔG_t (mS cm^{-2})	n
Control	22.9±2.0*	33.5±5.6*	14.7±4.2*	33.1±13.6	6–7
Quinine	8.0±0.9*,#	15.8±4.2*,#	3.8±0.8*,#	18.2±4.6*	6–7
Control	23.6±2.4*	34.9±4.4*	11.9±2.9*	11.7±4.3	5
TEA	20.5±3.4*	44.6±16.0*	9.6±2.7*	37.6±2.1	5
Control	17.2±1.6*	24.7±3.7*	13.0±1.7*	15.7±4.4*	7
Glibenclamide	15.0±2.3*	24.7±7.6*	7.0±1.6*,#	18.3±3.4*	7–8

Effect of nystatin (100 μg ml^{-1} at the mucosal side) on short-circuit current and tissue conductance in the absence of any drugs (control) and in the presence of glibenclamide (5×10^{-4} mol l^{-1} at the serosal side), quinine (10^{-3} mol l^{-1} at the serosal side) or TEA (tetraethylammonium chloride, 5×10^{-3} mol l^{-1} at the serosal side). Values are expressed as difference from the baseline (Δ I_{sc} and Δ G_t) before the administration of nystatin and are means±s.e.mean. *$P<0.05$ versus baseline; #$P<0.05$ versus control.

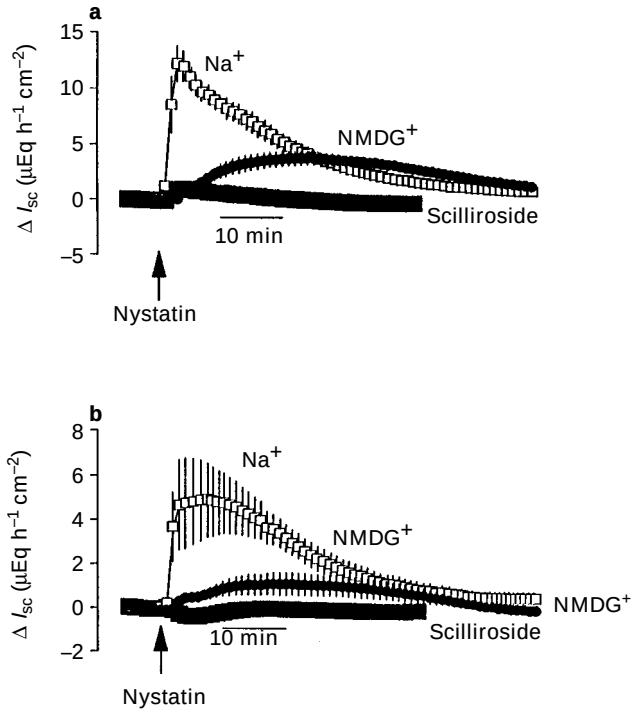

Figure 2 Effect of nystatin (100 μg ml^{-1} at the mucosal side) in the distal (a) and in the proximal colon (b) in the absence of a K^+ gradient (4.5 mmol l^{-1} at both sides), but in the presence of Na^+ (open squares), after substitution of Na^+ with N-methyl-D-glucamine ($NMDG^+$, solid circles), or in the presence of scilliroside (10^{-4} mol l^{-1} at the serosal side; open circles). Values are expressed as difference from the baseline just before administration of nystatin (Δ I_{SC}) and are means (symbols) and s.e.mean (vertical lines), $n=6-12$.

the proximal colon amounted to I_{SC} 2.6±0.2 μEq h^{-1} cm^{-2} at a G_t of 26.6±5.1 mS cm^{-2} ($n=16$). Increasing the mucosal K^+ concentration to 13.5 mmol l^{-1} (98 mmol l^{-1} NaCl/13.5 mmol l^{-1} KCl at the mucosal side) caused a marginal, transient increase in I_{SC} of 0.6±0.4 μEq h^{-1} cm^{-2} in the distal colon (not significant, $n=17$) and of 0.2±0.1 μEq h^{-1} cm^{-2} in the proximal colon ($P<0.05$, $n=16$), which soon fell to the former baseline value. Subsequent apical administration of nystatin (100 μg ml^{-1} at the mucosal side) stimulated a marked increase in I_{SC} (Figure 1a) by 23.4±1.2 μEq h^{-1} cm^{-2} in the distal ($P<0.05$, $n=17$) and by 13.0±2.0 μEq h^{-1} cm^{-2} in the proximal colon ($P<0.05$, $n=16$). The increase in I_{SC} was paralleled by a change in G_t (Figure 1b), which increased by 33.3±3.9 mS cm^{-2} ($P<0.05$, $n=17$) in the distal and by 22.0±6.1 mS cm^{-2} ($P<0.05$, $n=16$) in the proximal colon. The effect of nystatin on I_{SC} was significantly smaller in the proximal compared to the distal segment ($P<0.05$), whereas the difference in the G_t response did not reach statistical significance. In addition, the nystatin effect developed more slowly in the proximal colon. On average, it took 5.3±0.6 min ($n=17$) in the distal but 9.8±0.9 min in the proximal colon ($P<0.05$ versus distal colon, $n=16$) until a peak in I_{SC} was reached after administration of the ionophore. The concentration of nystatin (100 μg ml^{-1}) was a maximally effective one for the tissue used as investigated in detail by Pácha *et al.* (1991) and reproduced in our initial experiments.

In one experimental series, the K^+ concentration at the mucosal side was elevated to 111.5 mmol l^{-1}, which should better mimic the intracellular K^+ concentration after nystatin permeabilization. However, under these conditions nystatin induced very large currents which led to saturation of the voltage-clamp device. Consequently, the nystatin-induced current could not be investigated under 'more physiological' conditions, where the cytoplasmic side of the basolateral membrane faces a much higher K^+ concentration; an effect of a decreased intracellular K^+ concentration due to the efflux of K^+ into the mucosal compartment on, for example, basolateral K^+ channels can, therefore, not be excluded.

The increase in I_{SC} induced by nystatin was significantly inhibited in the presence of the K^+ channel blocker quinine (10^{-3} mol l^{-1} at the serosal side) in both colonic segments (Table 1). In contrast to quinine, the inhibitor of ATP-sensitive K^+ channels, glibenclamide (5×10^{-4} mol l^{-1} at the serosal side; Table 1), inhibited the nystatin response only in the proximal but not in the distal colon. Another K^+ channel blocker, tetraethylammonium (TEA; 5×10^{-3} mol l^{-1} at the serosal side; Table 1), was ineffective in both segments.

Contribution of the Na^+-K^+-pump to the nystatin-induced current

In order to estimate the contribution of the basolateral Na^+-K^+-pump to the nystatin-induced current, experiments were performed in the absence of a K^+ gradient, i.e. with standard 107 mmol l^{-1} NaCl/4.5 mmol l^{-1} KCl solution on both sides of the tissue. Under these conditions, nystatin (100 μg/ml^{-1} at the mucosal side) still induced an increase in I_{SC} of 12.1±1.0 μEq h^{-1} cm^{-2} in the distal (Figure 2a; $P<0.05$, $n=12$) and of 5.6±0.9 μEq h^{-1} cm^{-2} in the proximal colon (Figure 2b; $P<0.05$, $n=14$). Both effects were accompanied by an increase in G_t (Table 2). Both the increase in I_{SC} and that in G_t were significantly higher in the distal than in the proximal colon ($P<0.05$ for both parameters).

When Na^+ was substituted equimolarly by the impermeant cation, N-methyl-D-glucamine ($NMDG^+$), the increase in I_{SC} was nearly completely abolished (Figure 2a and b). The same result was obtained, when the tissue was pretreated with scilliroside, a potent inhibitor of the rat Na^+-K^+-ATPase (Robinson, 1970) (10^{-4} mol l^{-1} at the serosal side; Figure 2a and b), indicating that the nystatin-induced current in the absence of a K^+ gradient is caused by the Na^+-K^+-pump. This pump current was unaltered, when the tissue was pretreated with forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side; Table 2), suggesting that the activity of the Na^+-K^+-ATPase is

Table 2 Effect of nystatin in the absence of a K^+ gradient

	Distal colon		*Proximal colon*		
	ΔI_{sc} (μEq h^{-1} cm^{-2})	ΔG_t (mS cm^{-2})	ΔI_{sc} (μEq h^{-1} cm^{-2})	ΔG_t (mS cm^{-2})	n
Control	12.1±1.0*	14.9±2.7*	5.6±0.9*	4.6±1.9*	12–14
Bumetanide	16.4±2.0*	22.1±8.1*	6.1±1.3*	7.0±1.9*	6–7
Scilliroside	1.3±0.6#	5.7±2.8	0.1±0.2#	1.1±1.7	5–6
Na^+-free	4.1±0.6*,#	11.5±1.5*	1.2±0.5#	2.9±1.7	6
Bumetanide +Forskolin	9.5±2.8*	15.3±4.9*	4.2±2.2	11.0±7.9	6

Effect of nystatin (100 μg ml^{-1} at the mucosal side) on short-circuit current and tissue conductance in the absence of any drugs (control), in the presence of bumetanide (10^{-4} mol l^{-1} at the serosal side), forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side) + bumetanide or scilliroside (10^{-4} mol l^{-1} at the serosal side) and in the absence of Na^+. Values are expressed as difference from the baseline (ΔI_{sc} and ΔG_t) before the administration of nystatin and are means ± s.e.mean. *$P<0.05$ versus baseline, #$P<0.05$ versus response in the absence of inhibitors.

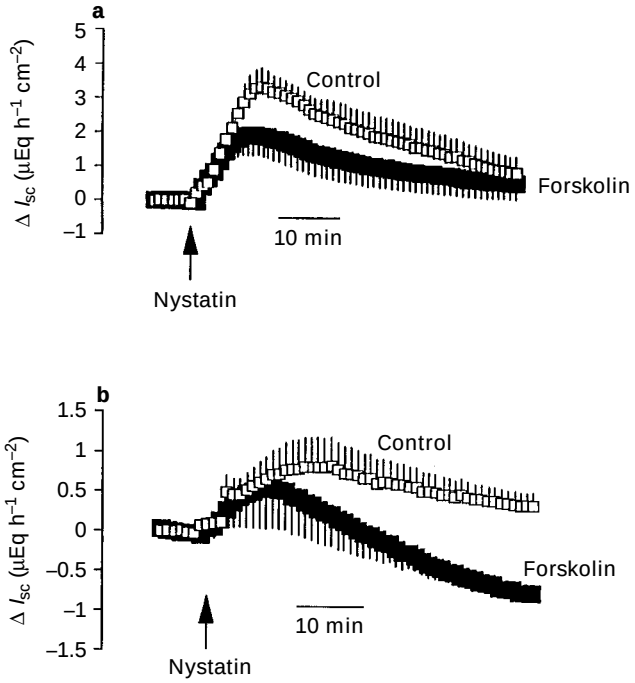

Figure 3 Effect of nystatin (100 μg ml^{-1} at the mucosal side) in the distal (a) and in the proximal colon (b) in the presence of a K^+ gradient (13.5 mmol l^{-1} at the mucosal and 4.5 mmol l^{-1} at the serosal side), the absence of Na^+ and the absence of drugs (control) or in the presence of forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side). Values are expressed as difference from the baseline just before the administration of nystatin (ΔI_{SC}) and are means (symbols) and s.e.mean (vertical lines), $n=6-8$.

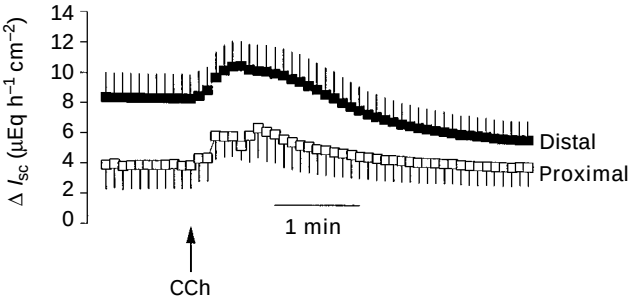

Figure 4 Carbachol (CCh 5×10^{-5} mol l^{-1} at the serosal side) stimulated the current across basolateral K^+ channels in the distal and the proximal colon. Carbachol was administered in the presence of a K^+ gradient (13.5 mmol l^{-1} at the mucosal and 4.5 mmol l^{-1} at the serosal side), scilliroside (10^{-4} mol l^{-1} at the serosal side) and of nystatin (100 μg ml^{-1} at the mucosal side), when the I_{SC} response induced by the ionophore started to decay. Values are means (symbols) and s.e.mean (vertical lines), $n=6-7$.

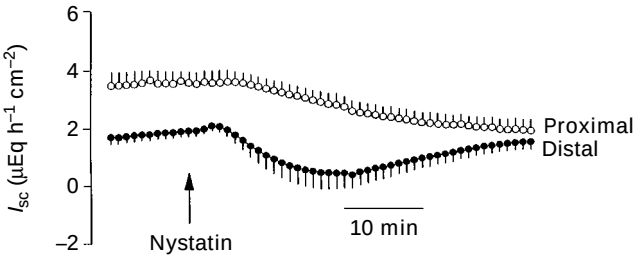

Figure 5 Effect of nystatin (100 μg ml^{-1} at the mucosal side) in the distal and in the proximal colon in the presence of a Cl^- gradient (107 mmol l^{-1} NaCl/4.5 mmol l^{-1} KCl at the mucosal and a 107 mmol l^{-1} NaGluc/4.5 mmol l^{-1} KGluc at the serosal side) and of scilliroside (10^{-4} mol l^{-1} at the serosal side). Values are means (symbols) and s.e.mean (vertical lines), $n=6-8$.

not regulated by cyclic AMP. The series of experiments with forskolin was performed in the presence of bumetanide (10^{-4} mol l^{-1} at the serosal side), an inhibitor of the basolateral Na^+-K^+-2 Cl^--cotransporter, in order to prevent forskolin-induced anion secretion. Bumetanide alone had no effect on the nystatin response (Table 2).

Contribution of K^+ conductances to the nystatin-induced current

In order to measure the current across basolateral K^+ conductances, the pump current was abolished either by Na^+-free media or by administration of scilliroside; the K^+ current was generated by administration of a K^+ gradient (13.5 mmol l^{-1} at the mucosal and 4.5 mmol l^{-1} at the serosal side). After blockade of the pump nystatin still induced an increase in I_{SC} of 3.5±0.5 μEq h^{-1} cm^{-2} (Figure 3a; $P<0.05$, $n=6$) in the distal and of 1.0±0.4 μEq h^{-1} cm^{-2} in the proximal colon (Figure 3b; $P<0.05$, $n=8$). The effect of nystatin was significantly greater in the distal compared to the proximal colon ($P<0.05$).

The maximal increase in I_{SC} induced by nystatin was significantly ($P<0.05$) inhibited in the distal colon, when the tissue was pretreated with forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side; Figure 3a). In order to prevent forskolin from inducing Cl^- secretion, these experiments were performed in the presence of bumetanide (10^{-4} mol l^{-1} at the serosal side). In the proximal colon, the maximal increase in I_{SC} evoked by nystatin was only significantly reduced by forskolin. However, the response was much less sustained (Figure 3b).

In contrast, carbachol (5×10^{-5} mol^{-1} at the serosal side) stimulated the K^+ current induced by nystatin (Figure 4). In this experimental series nystatin was administered in the presence of a K^+ gradient (13.5 mmol l^{-1} at the mucosal and

4.5 mmol l^{-1} at the serosal side) and in the presence of scilliroside (10^{-4} mol l^{-1} at the serosal side) to suppress the pump current. When the I_{SC} response evoked by nystatin started to decay, carbachol was administered. This cholinoreceptor agonist caused a prompt increase in I_{SC} of 2.8 ± 0.4 μEq h^{-1} cm^{-2} ($P < 0.05$, $n = 7$) in the distal colon (Figure 4) and of 3.6 ± 0.8 μEq h^{-1} cm^{-2} in the proximal colon (Figure 4; $P < 0.05$, $n = 6$).

Basolateral Cl^- conductance

Patch-clamp experiments have revealed the presence of volume-sensitive basolateral Cl^- channels in the rat distal colon (Diener *et al*., 1992). In order to find out whether this conductance may be activated after nystatin-permeabilization, nystatin was administered in the presence of a Cl^- gradient, i.e. in the presence of 107 mmol l^{-1} NaCl/4.5 mmol l^{-1} KCl solution at the mucosal and a 107 mmol l^{-1} NaGluc/4.5 mmol l^{-1} KGluc solution at the serosal side. The current carried by the Na^+-K^+-pump was blocked by administration of scilliroside (10^{-4} mol l^{-1} at the serosal side), current across basolateral K^+ conductances was avoided by a missing K^+ gradient. Under these conditions, nystatin induced a decrease in I_{SC} in the distal colon by -1.8 ± 0.4 μEq h^{-1} cm^{-2} ($P < 0.05$, $n = 8$; Figure 5). A significant ($P < 0.05$) decrease in I_{SC} was also evoked in the proximal colon, which developed more slowly compared to the distal colon (Figure 5) indicating the presence of a basolateral Cl^- conductance in both colonic segments.

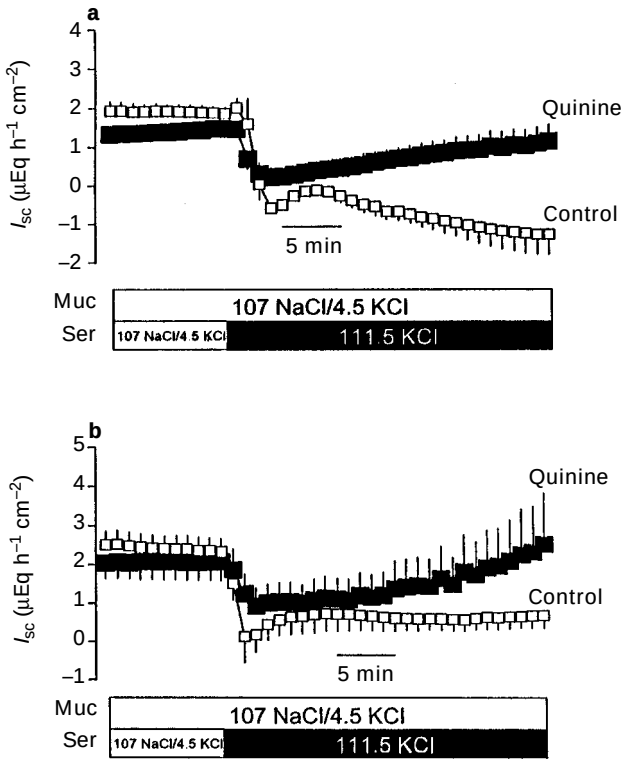

Figure 6 Effect of basolateral depolarization (111.5 mol l^{-1} KCl; solid bars) in the distal (a) and proximal (b) colon in the absence of any inhibitor (control) and in the presence of quinine (10^{-3} mol l^{-1} at the mucosal side). Values are means and s.e.mean (vertical lines), $n = 5-6$.

Current across apical K^+ conductances

The use of nystatin allows the measurement of ionic currents across the basolateral membrane. However, in partially stripped intestinal epithelium nystatin or other ionophores are not suitable to permeabilize the basolateral membrane, because these drugs do not reach the basolateral epithelial membrane in sufficient concentrations due to the presence of subepithelial tissue such as connective tissue or smooth muscle cells. Therefore, another approach was selected to measure currents across apical K^+ conductances and their putative regulation. The basolateral membrane was depolarized by a high K^+ solution (111.5 mmol l^{-1} KCl at the serosal side). Due to the high basolateral K^+ permeability, the electrical properties of the tissue, which are normally characterized by two batteries in series, are then expected to be dominated by the apical membrane (Fuchs *et al*., 1977).

Basolateral depolarization by a high K^+ solution induced a prompt decrease in I_{SC} in the distal and the proximal colon (Figure 6a and b). In the distal colon, the decrease in I_{SC} was biphasic, an initial decrease of -3.0 ± 0.2 μEq h^{-1} cm^{-2} was followed by a secondary decrease of -2.7 ± 0.3 ($P < 0.05$, $n = 6$) paralleled by an increase in G_t by 9.6 ± 2.7 mS cm^{-2} during the first and by 13.6 ± 2.4 mS cm^{-2} ($n = 6$, $P < 0.05$ for both) during the second peak. In contrast, in the proximal colon I_{SC} decreased transiently by -2.6 ± 0.5 μEq h^{-1} cm^{-2}, and then stabilized at a value of -1.9 ± 0.4 μEq h^{-1} cm^{-2} below the former baseline ($P < 0.05$, $n = 6$). During the maximal decrease in I_{SC} G_t increased by 9.4 ± 0.9 mS cm^{-2} ($P < 0.05$, $n = 6$). In both colonic segments, the I_{SC} response evoked by basolateral K^+ depolarization was significantly inhibited by the K^+ channel blocker quinine (10^{-3} mol l^{-1} at the mucosal side), indicating that this current is carried by K^+ flow across an apical K^+ conductance (Figure 6a and b, Table 3). Another K^+ channel blocker, tetraethylammonium (TEA, 5×10^{-3} mol l^{-1} at the mucosal side), was ineffective (Table 3) at inhibiting the basal K^+ current.

The effect of the basolateral K^+ depolarization was significantly increased in both colonic segments, if the animals were kept for 2 weeks on a NaCl-poor diet (Table 3), suggesting an upregulation of the apical K^+ conductance by a secondary hyperaldosteronism.

When the basolateral K^+ depolarization was performed in the absence of Cl^- (111.5 mmol^{-1} KGluc instead of 107 mmol l^{-1} NaGluc/4.5 mmol l^{-1} KGluc), a similar decrease in I_{SC} was evoked in both colonic segments, i.e. a decrease in I_{SC} by -2.2 ± 0.5 μEq h^{-1} cm^{-2} in the distal and by -2.3 ± 0.5 μEq h^{-1} cm^{-2} in the proximal colon ($P < 0.05$ for both, $n = 6$). Administration of carbachol (5×10^{-5} mol l^{-1} at

Table 3 Effect of basolateral K^+ depolarization (111.5 mmol l^{-1} K^+ at the serosal side)

	Δ I_{sc} (μEq h^{-1} cm^{-2})				
	Distal colon		*Proximal colon*		
	Peak 1	*Peak 2*	*Peak 1*	*Peak 2*	n
Control	-3.0 ± 0.2*	-2.7 ± 0.3*	-2.6 ± 0.5*	-1.9 ± 0.4*	6
TEA	-3.0 ± 0.1*	-4.2 ± 0.3*	-2.5 ± 0.4*	-1.3 ± 0.2*	6–8
Quinine	-1.2 ± 0.2*#	-0.6 ± 0.2*#	-1.2 ± 0.1*#	-1.0 ± 0.0*	5–6
Na^+-free diet	-3.9 ± 0.3*#	-3.2 ± 0.7*	-4.1 ± 0.3*#	-2.3 ± 0.2*	6

Effect of basolateral K^+ depolarization under control conditions and in the presence of TEA (tetraethylammonium chloride, 5×10^{-3} mol l^{-1} at the mucosal side) or quinine (10^{-3} mol l^{-1} at the serosal side) or after 2 weeks NaCl-poor diet. Peak 2 in the I_{sc} response was measured at the second maximal decrease in I_{sc} or, if not present as in the proximal colon, 15 min after administration of the K^+ gradient. Values are expressed as difference (Δ I_{sc}) from the baseline before the basolateral depolarization and are means $\pm$ s.e.mean. *$P < 0.05$ versus baseline; #$P < 0.05$ versus control response to basolateral depolarization.

the serosal side) under these conditions, i.e. in the absence of Cl^- and in the presence of a high basolateral K^+ concentration, stimulated a transient negative I_{SC} in both colonic segments (Figure 7a and b). This negative I_{SC} was nearly completely suppressed ($P<0.05$ for both colonic segments, $n=6$), if carbachol was administered in the presence of the K^+ channel blocker TEA (5×10^{-3} mol l^{-1} at the mucosal side; Figure 7a and b) indicating that carbachol opens an apical TEA-sensitive K^+ conductance.

In contrast to carbachol, the agonist of the cyclic AMP-pathway, forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side), was unable to stimulate a current across the apical K^+ conductance (Figure 8). This particular experiment was performed in a Cl^- and HCO_3^--free Tyrode solution, because in the presence of HCO_3^--forskolin still induced an increase in I_{SC}, probably due to stimulation of HCO_3^- secretion (data not shown). In order to determine whether the missing effect of forskolin on apical K^+ conductance may be caused by unspecific inhibition of the cyclic AMP-pathway under the experimental conditions, i.e. the K^+ depolarization, the ability of forskolin to open an apical Cl^- conductance was tested. Hence, forskolin was administered in the presence of a K^+- and a Cl^--gradient (107 mmol l^{-1} NaCl/4.5 mmol l^{-1} KCl at the mucosal side, 111.5 mmol l^{-1} KGluc at the serosal side). Under these conditions, forskolin induced a negative I_{SC} (Figure 9; $P<0.05$ for both colonic segments, $n=7-8$), which is what would be expected to occur if the drug opens an apical Cl^- conductance and thereby stimulates Cl^- flux from the mucosal to the serosal side along the applied chemical gradient.

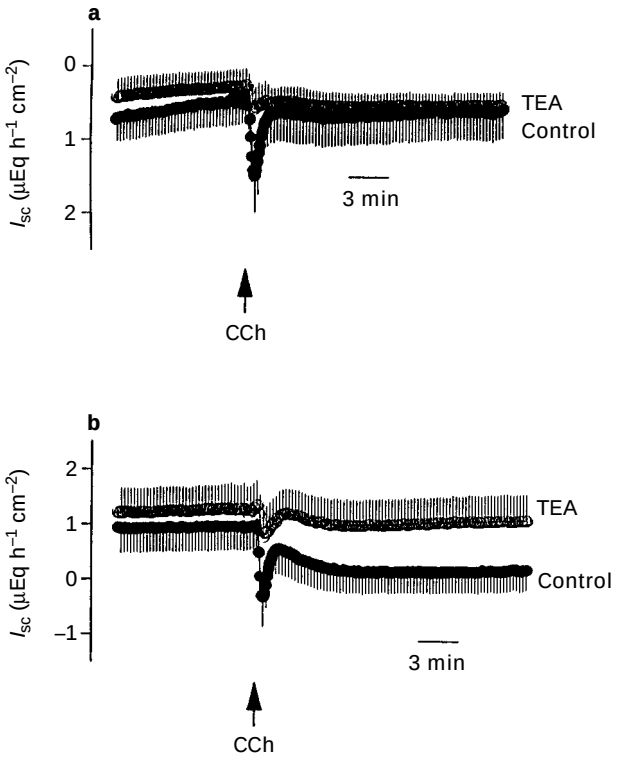

Figure 7 Carbachol (CCh, 5×10^{-5} mol l^{-1} at the serosal side) stimulated the current across apical K^+ channels in the distal (a) and the proximal (b) colon. Carbachol was administered in the presence of a K^+ gradient and in the absence of Cl^{-1} (111.5 mmol l^{-1} KGluc at the serosal side; 107 mmol l^{-1} NaGluc/4.5 mmol l^{-1} KGluc at the mucosal side), when the I_{SC} response induced by the basolateral K^+ depolarization had stabilized. The effect of carbachol was tested in the absence (control) and presence of TEA (5×10^{-3} mol l^{-1} at the mucosal side). Values are means (symbols) and s.e.mean. For the sake of graphical clarity, s.e.mean is presented as shaded area; $n=6$ in all groups.

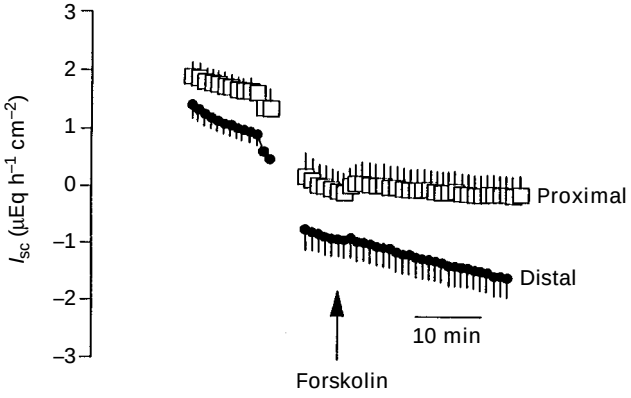

Figure 8 Forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side) did not stimulate the current across apical K^+ channels in the distal and the proximal colon. Forskolin was administered in the presence of a K^+ gradient and in the absence of Cl^- and HCO_3^- ions (145.4 mmol l^{-1} KGluc Tyrode at the serosal side; 140 mmol l^{-1} NaGluc/5.4 mmol l^{-1} KGluc Tyrode at the serosal side). Values are means (symbols) and s.e.mean (vertical lines), $n=7-8$.

Discussion

Permeabilization of the apical membrane by the ionophore, nystatin, induced a large increase in I_{SC} and G_t in the distal and in the proximal colon (Figure 1). This current is composed of two components, a pump current generated by the Na^+-K^+-ATPase, and a current across a basolateral K^+ conductance driven by the applied chemical K^+ gradient as shown by cation substitution experiments (Figure 2), sensitivity to scilliroside (Figure 2), and sensitivity to K^+ channel blockers (Table 1). The pump current, identified as scilliroside-sensitive, Na^+-dependent current in the absence of a K^+ gradient, was significantly greater in the distal than in the proximal colon. This is in accordance with the general transport properties of the proximal colon, which is equipped with a more leaky epithelium, as indicated by the higher G_t, so that a smaller part of the transported Na^+ has to pass the epithelium transcellularly involving the Na^+-K^+-ATPase. A similar result has been obtained by Sandle (1991) in nystatin-permeabilized rat colonic mucosa. The pump current was unaltered by pretreatment of the tissue with forskolin, an activator of the adenylate cyclase (Table 2). This suggests that the activity of the Na^+-K^+-ATPase is not regulated by cyclic AMP in the rat colon, although a regulation by a cyclic AMP-dependent phosphorylation has been observed in several tissues (Beguin *et al.*, 1994).

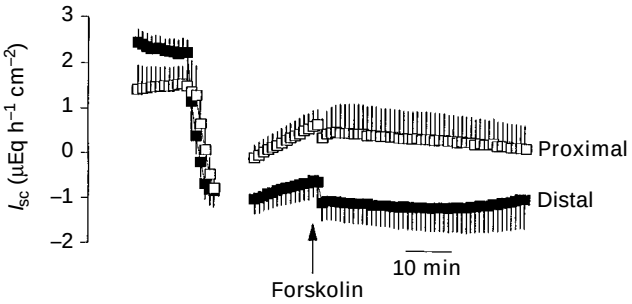

Figure 9 Forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side) stimulated the current across apical Cl^- channels in the distal and the proximal colon. Forskolin was administered in the presence of a K^+- and Cl^--gradient (107 mmol l^{-1} NaCl/4.5 mmol l^{-1} KCl at the mucosal side, 111.5 mmol l^{-1} KGluc at the serosal side). Values are means (symbols) and s.e.mean (vertical lines), $n=7-8$.

When the pump current was blocked by the use of Na^+-free solution or by administration of scilliroside, the current across the basolateral K^+ conductance could be investigated with a chemical K^+ gradient (mucosal : serosal K^+ concentration = 3 : 1) as driving force. As in the case of the current generated by the Na^+-K^+-ATPase, this I_{SC} was significantly greater in the distal than in the proximal colon (Figure 3) suggesting a higher basolateral K^+ conductance in the distal colon. This is consistent with the assumed main function of the basolateral K^+ channels, i.e. creating a bypass for K^+ pumped out of the cell by the Na^+-K^+-ATPase for recycling across the basolateral membrane. Due to the higher pump activity in the distal colon, a greater basolateral K^+ conductance is necessary in this colonic segment.

The increase in I_{SC} induced by nystatin is quite short-lived, i.e. it declines within about 45 min to baseline values. This time course has already been observed in other laboratories with the rat colon (J. Pácha, personal communication). Several reasons for this phenomenon are possible. The pump current may decline due to a reduction of intracellular ATP after permeabilization of the apical membrane. The current across the basolateral K^+ conductance may decline due to a reduction of the chemical K^+ gradient due to the diffusion of K^+ across the (cation selective) paracellular shunt. In addition, changes in cell volume after application of nystatin are quite likely to occur as shown by the opening of the volume-sensitive basolateral Cl^- conductance (Figure 5). The concomitant increase in cell volume will increase the paracellular resistance and therefore cause a decrease in G_t, which falls to baseline values within 45 min in distal colon, but stabilizes at a value of about 50% higher than the former baseline in the proximal colon. In addition, the time course of the nystatin response differed along the longitudinal axis of the colon, i.e. the increase in I_{SC} developed more slowly in the proximal compared to the distal colon. The reasons for this difference can only be speculated about. Nystatin interacts with sterols in the membrane in order to form pores (for review see e.g. Akaike & Harata, 1994). In guinea-pig colon, for example, differences in the regional content of cholesterol in the apical membrane have been demonstrated (Luciano *et al*., 1989). Consequently, differences in the lipid composition might be responsible for the slower time course of the nystatin response in the proximal colon.

The basolateral K^+ conductance is increased by the cholinoceptor agonist carbachol (Figure 4). Carbachol induced an increase in I_{SC} driven by a K^+ gradient in both the distal and the proximal colon. Carbachol has been shown to induce a rise in the intracellular Ca^{2+} concentration in the rat colonic epithelium (Diener *et al*., 1991), which activates a 16 pS basolateral K^+ channel (Bleich *et al*., 1996). The resulting hyperpolarization is the driving force for an increase in Cl^- secretion (Böhme *et al*., 1991; Strabel & Diener, 1995).

In contrast to the agonist of the Ca^{2+} pathway, forskolin, an agonist of the cyclic AMP pathway, causes inhibition of the I_{SC} across the basolateral K^+ conductance (Figure 3). This inhibition differs in time course in the distal and in the proximal colon. Whereas in the distal colon the early current response to nystatin is reduced in the presence of forskolin, forskolin inhibits only the current in the late phase of the nystatin response in the proximal colon, which may suggest a different regulation or mechanism with different types of K^+ channels in both colonic segments. The inhibition of the basolateral K^+ current by forskolin is in accordance with recent whole-cell patch-clamp experiments and recent efflux experiments, which demonstrated inhibition of a K^+ current by forskolin in the absence of Cl^- (Diener *et al*., 1996). At first glance, this inhibition seems to be surprising, because a decreased cellular K^+ conductance will limit epithelial Cl^- conductance due to a reduced driving force for Cl^- exit across apical Cl^- channels. In addition, Warth *et al*. (1996) have demonstrated the activation of a small conductance (< 3 pS) basolateral K^+ channel by forskolin in rat colonic crypts. However, recently the same group observed the simultaneous inhibition of basolateral Ca^{2+}-activated K^+ channels by forskolin, which has been attributed to a decrease in the intracellular Ca^{2+} concentration in the presence of this drug (Bleich *et al*., 1996). Consequently, in intact tissue (Figure 3), as in the intact cell measured by the whole-cell patch-clamp technique (Diener *et al*., 1996), the inhibitory action of forskolin on K^+ conductance seems to predominate.

However, inhibition of a K^+ conductance after stimulation of cyclic AMP-dependent protein phosphorylation has also been observed in other intestinal systems. In human distal colon, forskolin inhibits an aldosterone-activated K^+ conductance (Maguire *et al*., 1995). In crypt cells from guinea-pig distal colon, calyculin A, an inhibitor of protein phosphatases, which indirectly enhances cyclic AMP-dependent phosphorylation due to inhibition of dephosphorylation, inhibits basal K^+ influx into the cells (Del Castillo & Sepúlveda, 1995). The same has been observed with genistein (Diener & Hug, 1996), which is thought to act as a protein phosphatase inhibitor (Illek *et al*., 1996) and with calyculin A in the rat distal colon (Schultheiß & Diener, unpublished observations). In colonic carcinoma cell lines such as HT29-cl. 19A or T84-cells either no increase (Bajnath *et al*., 1991) or a decrease in basolateral K^+ conductance after stimulation of cyclic AMP-production have been found (Reenstra, 1993). This decrease in K^+ conductance is clearly contraproductive for the inducement of Cl^- secretion, which is one of the dominant epithelial responses after an elevation of the intracellular cyclic AMP concentration. However, a decrease in basolateral K^+ conductance may well explain the long-known inhibition of K^+ absorption by cyclic AMP, as discussed in detail recently (Diener *et al*., 1996).

A different approach was used to study the putative regulation of the apical K^+ conductance. The basolateral membrane was depolarized by a high K^+ buffer. Due to the high basolateral K^+ permeability, the electrical properties of the tissue, which are normally determined by two batteries, i.e. two membranes, in series, are then expected to be dominated by the apical membrane (Fuchs *et al*., 1977). Basolateral K^+ depolarization induced a negative I_{SC} in both the distal and proximal colon, which was blocked by mucosal administration of the K^+ channel blocker, quinine (Figure 6), indicating that this current is indeed carried by K^+ ions passing through an apical K^+ conductance driven by the chemical K^+ gradient. However, there was a difference in the time course of the depolarization-induced current in both segments: in the proximal colon the negative I_{SC} increased to a maximal value and then stabilized at a lower level, whereas in the distal colon stimulation was clearly biphasic, a fast activation within a few minutes was followed by a slower one, which increased steadily during 25–30 min (Figure 6). The reasons for the second phase of the I_{SC} response can only be speculated, it might indicate the presence of a K^+ conductance, which is slowly activated by depolarization, or of a volume-sensitive K^+ conductance activated due to cell swelling in the high K^+ buffer.

The current across the apical K^+ conductance is stimulated by carbachol (Figure 7a and b). Carbachol causes a short-lasting decrease in I_{SC} after basolateral K^+ depolarization. This response is nearly suppressed in the presence of TEA. However, this K^+ channel blocker is ineffective in reducing the K^+ current under basal conditions (Table 3), indicating the opening of a type of apical K^+ channel distinct from those which are active under basal conditions. In contrast to carbachol, forskolin, the agonist of the cyclic AMP-pathway, did not stimulate a current across the apical K^+ conductance (Figure 8). There was no evidence for stimulation of a negative I_{SC} in the presence of a high basolateral K^+ concentration by forskolin. However, control experiments revealed that the regulation of apical Cl^- channels by cyclic AMP was still intact, because in the presence of a K^+ together with a Cl^- gradient, forskolin was able to stimulate a negative I_{SC} consistent with a flow of Cl^- from the mucosal to serosal side along its chemical gradient: a response, which was more pronounced in the distal than in the proximal colon (Figure 9). This negative I_{SC} was quite small compared to the I_{SC} response

evoked by forskolin in intact tissue, an effect which may be explained by the small (but present) basolateral Cl^- conductance (Figure 5) limiting transepithelial Cl^- flow along the applied chemical gradient.

Finally, the regulation of the apical K^+ conductance by aldosterone was studied. Secondary hyperaldosteronism, induced by a NaCl-poor diet, stimulates the current across the apical K^+ conductance in both colonic segments (Table 3). Hyperaldosteronism has been shown to stimulate K^+ secretion (Sweiry & Binder, 1989). The present experiments demonstrate that this effect is not only caused by an increased driving force for K^+ exit across spontaneously open apical K^+ channels due to the depolarization caused by the induction of apical Na^+ channels, but is in addition due to the fact that the number of channels and/or their open time is increased. The aldosterone effect on the K^+ channels is restricted to the cells at the surface and the upper part of the crypt (Lomax *et al*., 1994).

Consequently, these data suggest that an increase in the intracellular Ca^{2+} concentration opens both an apical and a basolateral K^+ conductance. This effect will indirectly support Cl^- secretion due to the basolateral hyperpolarization and at the same time induce K^+ secretion due to the increased apical K^+ permeability. However, an increase in the intracellular cyclic AMP concentration seems to reduce total basolateral K^+ conductance. This will inhibit K^+ absorption, which is thought to involve exit of K^+ across basolateral K^+ channels (for review see Binder & Sandle, 1994) and indirectly stimulate K^+ secretion across spontaneously open K^+ channels, due to an increased apical to basolateral K^+ permeability ratio.

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References

AKAIKE, N. & HARATA, N. (1994). Nystatin perforated patch recording and its application to analyses of intracellular mechanisms. *Jpn. J. Physiol.*, **44,** 433–473.

ANDRES, H., BOCK, R., BRIDGES, R.J., RUMMEL, W. & SCHREINER, J. (1985). Submucosal plexus and electrolyte transport across rat colonic mucosa. *J. Physiol.*, **364,** 301–312.

BAJNATH, R.B., AUGERON, C., LABOISSE, C.L., BIJMAN, J., DE JONGE, H.R. & GROOT, J.A. (1991). Electrophysiological studies of forskolin-induced changes in ion transport in the human colon carcinoma cell line HT-29.cl.19A: Lack of evidence for a cAMP-activated basolateral K^+ conductance. *J. Memb. Biol.*, **122,** 239–250.

BEGUIN, P., BEGGAH, A.T., CHIBALIN, A.V., BURGENER-KAIRUZ, P., JAISSER, F., MATHEWS, P.M. ROSSIER, B.C., COTECCHIA, S. & GEERING, K. (1994) Phosphorylation of the Na, K-ATPAse alpha-subunit by protein kinase A and C in vitro and in intact cells. Identification of a novel motif for PKC-mediated phosphorylation. *J. Biol. Chem.*, **269,** 24437–24445.

BINDER, H.J. & SANDLE, G.J. (1994). Electrolyte transport in the mammalian colon. In: *Physiology of the Gastrointestinal Tract*, 3. ed. Johnson L.R., pp. 2133–2171. New York: Raven Press.

BLEICH, M., RIEDEMANN, N., WARTH, R., KERSTAN, D., LEIPZIGER, J., HÖR, M., VAN DRIESSCHE, W. & GREGER, R. (1996). Ca^{2+} regulated K^+ and nonselective cation channels in the basolateral membrane of rat colonic crypt base cells. *Pflügers Arch.*, **432,** 1011–1022.

BÖHME, M., DIENER, M. & RUMMEL, W. (1991). Calcium- and cyclic-AMP-mediated secretory responses in isolated colonic crypts. *Pflügers Arch.*, **419,** 144–151.

DEL CASTILLO, J.R. & SEPÚLVEDA, F.V. (1995). Activation of an $Na^+/K^+/2Cl^-$ cotransport system by phosphorylation in crypt cells isolated from guinea pig distal colon. *Gastroenterology*, **109,** 387–396.

DIENER, M., EGLÈME, C. & RUMMEL, W. (1991). Phospholipase C-induced anion secretion and its interaction with carbachol in the rat colonic mucosa. *Eur. J. Pharmacol.*, **200,** 267–276.

DIENER, M. & HUG, F. (1996). Modulation of chloride secretion in rat distal colon by genistein, a protein tyrosine kinase inhibitor. *Eur. J. Pharmacol.*, **299,** 161–170.

DIENER, M., HUG, F., STRABEL, D. & SCHARRER, E. (1996). Cyclic AMP-dependent regulation of K^+ transport in the rat distal colon. *Br. J. Pharmacol.*, **118,** 1477–1487.

DIENER, M., NOBLES, M. & RUMMEL, W. (1992). Activation of basolateral Cl^- channels in the rat colonic epithelium during regulatory volume decrease. *Pflügers Arch.*, **421,** 530–538.

FOSTER, E.S., SANDLE, G.I., HAYSLETT, J.P. & BINDER, H.J. (1983). Cyclic adenosine monophosphate stimulates active potassium secretion in the rat colon. *Gastroenterology*, **84,** 324–330.

FUCHS, W., LARSEN, E.H. & LINDEMANN, B. (1977). Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol.*, **267,** 137–166.

ILLEK, B., FISCHER, H. & MACHEN, T.E. (1996). Alternate stimulation of apical CFTR by genistein in epithelia. *Am. J. Physiol.*, **270,** C265–C275.

LINDSTRÖM, C.G., ROSENGREN, J.-E. & FORK, F.-T. (1979). Colon of the rat. An anatomic, histologic and radiographic investigation. *Acta Radiol. Diagn.*, **20,** 523–536.

LOMAX, R.B., MCNICHOLAS, C.M., LOMBES, M. & SANDLE, G.I. (1994). Aldosterone-induced apical Na^+ and K^+ conductances are located predominantly in surface cells in rat distal colon. *Am. J. Phsyiol.*, **266,** G71–G82.

LUCIANO, L., KONITZ, H. & REALE, E. (1989). Localization of cholesterol in the colonic epithelium of the guinea pig: regional differences and function implications. *Cell Tissue Res.*, **258,** 339–347.

MAGUIRE, D., O'SULLIVAN, G. & HARVEY, B.J. (1995). Membrane and genomic mechanisms for aldosterone effect in human colon. *Surg. Forum.*, **46,** 203–205.

MCCABE, R.D. & SMITH, P.L. (1985). Colonic potassium and chloride secretion: role of cAMP and calcium. *Am. J. Physiol.*, **248,** C103–C109.

MCCABE, R.D., SMITH, P.L. & SULLIVAN, L.P. (1986). Ion transport by rabbit descending colon: mechanisms of transepithelial potassium transport. *Am. J. Physiol.*, **250,** C486–C494.

PÁCHA, J., TEISINGER, T., POPP, M. & CAPEK, K. (1991). Na, K-ATPase and the development of Na^+ transport in rat distal colon. *J. Membr. Biol.*, **120,** 201–210.

PARSONS, D.S. & PATERSON, C.R. (1965). Fluid and solute transport across rat colonic mucosa. *Quart. J. Exp. Physiol.*, **50,** 220–231.

REENSTRA, W.W. (1993). Inhibition of cAMP- and Ca-dependent Cl^- secretion by phorbol esters: inhibition of basolateral K^+ channels. *Am. J. Physiol.*, **264,** C161–C168.

ROBINSON, J.W.L. (1970). The difference in sensitivity to cardiac steroids of $(Na^+\text{-}K^+)$-stimulated ATPase and amino acid transport in the intestinal mucosa of the rat and other species. *J. Physiol.*, **206,** 41–60.

SANDLE, G.I. (1991). Segmental variability of glucocorticoid induced electrolyte transport in rat colon. *Gut*, **32,** 936–940.

STRABEL, D. & DIENER, M. (1995). Evidence against direct activation of chloride secretion by carbachol in the rat distal colon. *Eur. J. Pharmacol.*, **274,** 181–191.

SWEIRY, J.H. & BINDER, H.J. (1989). Characterization of aldosterone-induced potassium secretion in rat distal colon. *J. Clin. Invest.*, **83,** 844–851.

WARTH, R., RIEDEMANN, N., BLEICH, M., VAN DRIESSCHE, W. & GREGER, R. (1996). The cAMP-regulated and 293B-inhibited K^+ conductance of rat colonic crypt base cells. *Pflügers Arch.*, **432,** 81–88.

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