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Basolateral potassium channels of rabbit colon epithelium: role in sodium absorption and chloride secretion

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Abstract

In order to assess the role of different classes of K^+ channels in recirculation of K^+ across the basolateral membrane of rabbit distal colon epithelium, the effects of various K^+ channel inhibitors were tested on the activity of single K^+ channels from the basolateral membrane, on macroscopic basolateral K^+ conductance, and on the rate of Na^+ absorption and Cl^- secretion. In single-channel measurements using the lipid bilayer reconstitution system, high-conductance (236 pS), Ca^{2+}-activated K^+ (BK_{Ca}) channels were most frequently detected; the second most abundant channel was a low-conductance K^+ channel (31 pS) that exhibited channel rundown. In addition to Ba^{2+} and charybdotoxin (ChTX), the BK_{Ca} channels were inhibited by quinidine, verapamil and tetraethylammonium (TEA), the latter only when present on the side of the channel from which K^+ flow originates. Macroscopic basolateral K^+ conductance, determined in amphotericin-permeabilised epithelia, was also markedly reduced by quinidine and verapamil, TEA inhibited only from the lumen side, and serosal ChTX was without effect. The chromanol 293B and the sulphonylurea tolbutamide did not affect BK_{Ca} channels and had no or only a small inhibitory effect on macroscopic basolateral K^+ conductance. Transepithelial Na^+ absorption was partly inhibited by Ba^{2+}, quinidine and verapamil, suggesting that BK_{Ca} channels are involved in basolateral recirculation of K^+ during Na^+ absorption in rabbit colon. The BK_{Ca} channel inhibitors TEA and ChTX did not reduce Na^+ absorption, probably because TEA does not enter intact cells and ChTX is 'knocked off' its extracellular binding site by K^+ outflow from the cell interior. Transepithelial Cl^- secretion was inhibited completely by Ba^{2+} and 293B, partly by quinidine but not by the other K^+ channel blockers, indicating that the small (<3 pS) K_VLQT1 channels are responsible for basolateral K^+ exit during Cl^- secretion. Hence different types of K^+ channels mediate basolateral K^+ exit during transepithelial Na^+ and Cl^- transport.

Keywords: Rabbit colon epithelium; Basolateral membrane potassium conductance; Amphotericin B; Quinidine; Verapamil; 293B

1. Introduction

The basolateral membrane of epithelial cells contains K^+ channels which are primarily responsible for the conductance of this membrane. The combination of the Na^+/K^+-exchange pump with the K^+ leak in the basolateral membrane accounts for both the characteristic intracellular ion composition (low Na^+ and high K^+ concentrations) and the electrical membrane potential difference, thereby creating the driving forces for apical Na^+ entry and Cl^- exit [1,2]. Basolateral K^+ recirculation is thought to be an absolute requirement for both Na^+ absorption and Cl^- secretion; variations in transepithelial transport of

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both ions must be accompanied by proportional changes in basolateral K^+ outflow [3,4].

But whereas the apical Na^+ entry and Cl^- exit mechanisms are well characterised, it is open which class(es) of K^+ channels take part in recirculation of K^+ across the basolateral membrane during transepithelial Na^+ absorption and Cl^- secretion. This uncertainty may be related to the fact that K^+ channels comprise the most diverse family of ion channels [3–5]. The present experiments were designed to define the types of K^+ channels that are responsible for basolateral K^+ exit during Na^+ absorption and Cl^- secretion in rabbit colon epithelium using microscopic (single-channel) and macroscopic measurements of the basolateral K^+ conductance.

2. Materials and methods

2.1. Single-channel measurements

Rabbits were killed by a blow on the head followed by exsanguination[1]. From the epithelium of descending colon a highly enriched fraction of basolateral plasma membrane vesicles (BLMV) was produced according to Wiener and coworkers [6]. The BLMV were suspended in 250 mM sucrose and 10 mM HEPES/Tris, pH 7.2, stored at −80°C and thawed immediately prior to use. K^+ channels present in this preparation, which is essentially free of membranes from non-epithelial cells, were reconstituted into planar lipid bilayers consisting of phosphatidylethanolamine and phosphatidylserine as described earlier [7]. Briefly, basolateral membrane vesicles were fused at room temperature with a bilayer that occluded the aperture (diameter 180–240 μm) of a Teflon septum (thickness 12 μm) which was mounted vertically between two halves of a Teflon chamber (volume 1.5 ml on each side). The *cis* solution, to which the BLMV were added, contained initially 150 mM KCl and the *trans* solution 5 mM KCl, both in 10 mM HEPES/Tris, pH 7.2, and 250 μM $CaCl_2$. After a channel had appeared, free Ca^{2+} was lowered to 28 μM in most experiments by addition of K^+-EGTA [7]. Rapid mixing of added compounds was achieved with magnetic stirrers in both chamber halves.

The *cis* and *trans* solutions were connected to a patch-clamp amplifier (EPC-7, HEKA, Lambrecht/Pfalz, Germany) via 0.5 M KCl-agar bridges and Ag/AgCl electrodes. V_m, the 'holding' voltage across the bilayer, is defined as the electrical potential of the *cis* compartment with reference to the *trans* (ground) compartment. Single-channel current, recorded on a video cassette recorder, was filtered (usually at 500 Hz) by an eight-pole low-pass Bessel filter and analysed with the pCLAMP program (Axon Instruments, Foster City, CA, USA). 30–40 s of channel activity were analysed at each holding voltage.

2.2. Measurement of macroscopic basolateral conductance

The properties of the basolateral membrane of rabbit descending colon epithelium were studied using the technique of selective luminal membrane permeabilisation with the pore-forming antibiotic amphotericin B [8,9]. Isolated colon epithelia, prepared by blunt dissection, were mounted in Ussing chambers and short-circuited with an automatic voltage clamp (VC600, Physiologic Instruments, San Diego, CA, USA). The tissue area exposed in the chambers was 1.13 cm^2. The luminal bathing solution was composed of (mM) 119 K^+-gluconate, 21 $KHCO_3$, 2.4 K_2HPO_4, 0.6 KH_2PO_4, 1.2 Ca^{2+}-acetate, 1.2 Mg^{2+}-acetate and 10 D-glucose. Hence, the luminal solution contained a high K^+ concentration to mimic intracellular K^+ levels but was free of Na^+ (to exclude a contribution of the electrogenic Na^+/K^+-ATPase to the basolateral membrane potential) and Cl^- (to prevent cell swelling after permeabilisation of the luminal membrane). The composition of the serosal bathing solution was identical to that of the high-K^+ luminal solution except that Na^+ replaced K^+ in the gluconate and bicarbonate salts. The solutions were kept at 37°C in the Ussing chambers and recirculated by a gas lift system with 95% O_2 and 5% CO_2, resulting in a pH of 7.4.

Amphotericin B was added to the luminal solution to give a final concentration of 10 μg/ml (10.8 μM). In the presence of amphotericin the transepithelial voltage, V, was clamped to −40 mV (luminal solution with respect to the serosal solution) to imitate

[1] Laboratory Animals 30 (1996) 293–316, and 31 (1997) 1–32.

the physiological electrical potential difference across the basolateral membrane [10]. The short-circuit current, I_{sc}, was measured every minute by changing V to zero for 2 s. The transepithelial (chord) conductance, G_t, was calculated from the difference of the transepithelial current, ΔI, at −40 and 0 mV, $G_t = \Delta I/\Delta V$.

When the effect of amphotericin on tissue conductance and transepithelial current had stabilised (this was the case approx. 30 min after addition of amphotericin), transepithelial current–voltage (I–V) relations were obtained by generating a train of square voltage pulses of 2 s duration and monitoring the corresponding currents. Starting from −40 mV, the transepithelial voltage was clamped from −120 to +40 mV in 20 mV increments with a return to −40 mV between each step. A positive current represents cation flow from the luminal to the serosal side of the epithelium.

2.3. Transepithelial Na^+ absorption and Cl^- secretion

The effects of various K^+ channel blockers on the rate of Na^+ absorption and Cl^- secretion across rabbit descending colon epithelium were studied in the short-circuit apparatus with NaCl Ringer (composition in mM: 119 NaCl, 21 $NaHCO_3$, 2.4 K_2HPO_4, 0.6 KH_2PO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$ and 10 D-glucose) on both sides of the tissue [11,12].

2.4. Materials

293B was generously provided by Dr. R. Greger (University of Freiburg, Germany), amphotericin B by Bristol Myers Squibb, and amiloride by Merck, Sharp and Dohme. Phosphatidylethanolamine and phosphatidylserine were obtained from Avanti Polar Lipids (Alabaster, AL, USA), and charybdotoxin and tetrodotoxin from Alomone Laboratories (Jerusalem, Israel). All other chemicals were from local suppliers.

239B, clotrimazole and tolbutamide were dissolved in dimethyl sulphoxide (DMSO). The final DMSO concentrations never exceeded 0.2% in single-channel or Ussing chamber experiments. These DMSO concentrations were without effect.

2.5. Statistics

Results are given as means ± standard deviation (S.D.) unless stated otherwise based on n, the number of experiments. The statistical significance of a difference between means was calculated using the paired or unpaired t-test, as appropriate. Linear and non-linear regressions were determined by the least-squares method.

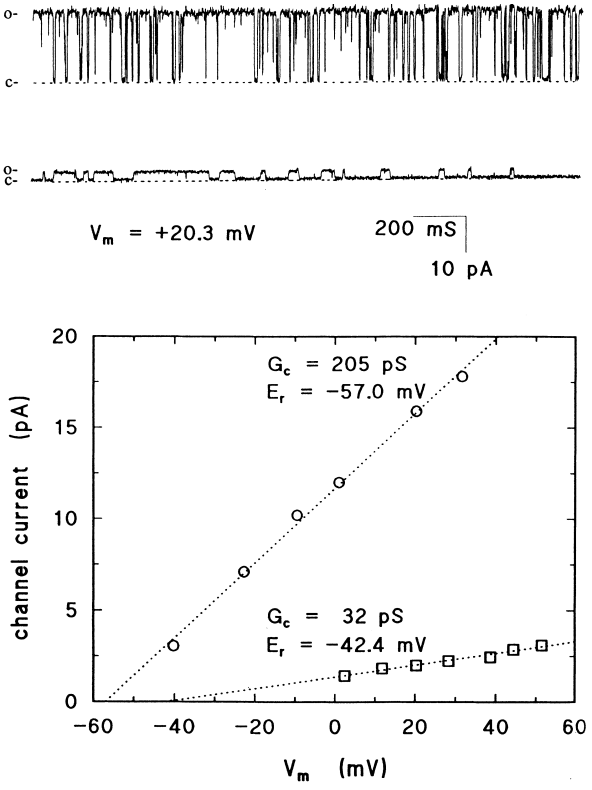

Fig. 1. Examples of the current through two K^+ channels from the basolateral membrane of rabbit colonocytes reconstituted in planar phospholipid bilayers (top) and current–voltage relations of these channels (bottom) with initial KCl concentrations of 150 mM *cis* and 5 mM KCl *trans*. The current through the high-conductance channel was filtered at 500 Hz, that through the low-conductance channel at 100 Hz. V_m, voltage across the bilayer; G_c, channel conductance; E_r, reversal potential; o and c, open (conductive) and closed (non-conductive) states of the channels.

3. Results

3.1. Single-channel experiments

When basolateral membrane vesicles from rabbit colon epithelium were fused with planar lipid bilayers, two types of K^+ channels were identified, one with a high conductance and one with a low conductance (Fig. 1). In some instances, large and small channels were observed simultaneously in the same bilayer. The reversal potential, E_r, of the large channel is close to the Nernst potential of −60 mV, calculated from the *cis*:*trans* gradient of K^+ activity of 111.0 ± 2.0 ($n = 47$) and 10.3 ± 0.5 mM ($n = 50$), measured at the conclusion of the experiments by flame photometry and correction with the activity coefficients[2] [7]. E_r of the small channel was considerably lower than the Nernst potential.

The frequency distribution of the conductance, G_c, of all basolateral K^+ channels reconstituted in planar bilayers is shown in Fig. 2. Clearly, the reconstituted K^+ channels varied widely in conductance, but most channels were of the high-conductance type with a mean G_c of 236 ± 29 pS ($n = 241$); the mean E_r of these channels was -56.3 ± 11.5 mV. Hence, the permeability ratio of this channel for K^+ over Cl^-, P_K/P_{Cl}, was 30, calculated from the biionic diffusion potential. A second cluster of channels was observed at low-conductance values. These small K^+ channels had a mean G_c of 31 ± 15 pS ($n = 39$) and an E_r of -36.7 ± 16.5 mV. Therefore, the P_K/P_{Cl} ratio of the low-conductance K^+ channel was only 4.3.

The high-conductance K^+ channel is activated by Ca^{2+} and depolarisation of the transmembrane electrical potential difference, and inhibited by charybdotoxin (ChTX) and Ba^{2+} [13,14]. The small K^+ channel exhibited 'channel rundown' (i.e. a decrease in activity with time), therefore it was not possible to test the effects of potential K^+ channel inhibitors systematically on this channel. However, it seems that the small K^+ channel is not dependent on Ca^{2+} and not blocked by ChTX (data not shown).

The current through the high-conductance, Ca^{2+}-activated (BK_{Ca}) channels of colon epithelium was transformed by quinidine into a rapidly fluctuating noise pattern in a concentration-dependent manner (Fig. 3). Hence, the interaction of quinidine with this channel appears to be that of a rapidly dissociating block [15], similar to its effect on BK_{Ca} channels from pig pancreatic acinar cells [16]. A rapidly fluctuating block of the rabbit colon BK_{Ca} channel was also observed with verapamil, an inhibitor of the delayed rectifier K^+ channel in epithelial, cardiac and neuronal cells [17–19]. The intensity of the blocking effect of verapamil was also concentration-dependent (Fig. 3). Both agents were effective from the intra- and extracellular side of the channel, consistent with their high lipophilicity, but while with quinidine open and closed states of the channel were still discernible, the rapid current fluctuations induced by verapamil precluded detection of the open state, i.e. the life time of the open state was reduced below the resolution of our recording system. This effect of verapamil is similar to that of gallopamil (D600, the methoxy derivative of verapamil) on BK_{Ca} channel activity of rat aortic myocytes [20].

Tetraethylammonium (TEA), a blocker of BK_{Ca} channels in a variety of cells [21–23], decreased colonic BK_{Ca} channel current and conductance in a concentration-dependent manner but only when present on the *cis* side of the membrane, i.e. the side from which K^+ flow originates; from the *trans* side TEA was without effect. The open state probability, P_o, of the channel was not changed by TEA

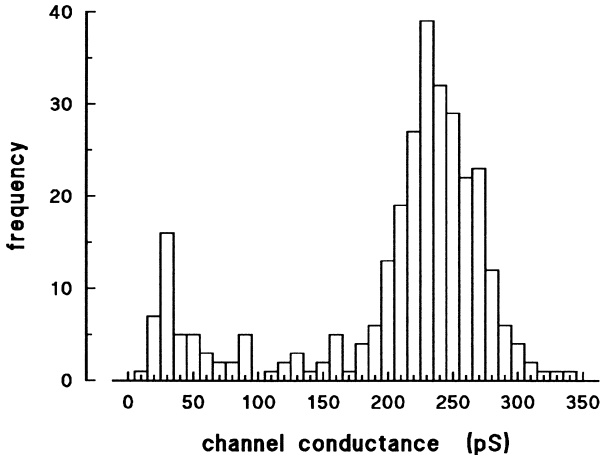

Fig. 2. Frequency distribution of the conductance of K^+ channels from the basolateral membrane of rabbit colonocytes reconstituted in planar lipid bilayers. Total number of channels: 301.

[2] The K^+ activity in the *trans* solution increased during the course of the experiment because the bilayer was ruptured and reformed several times.

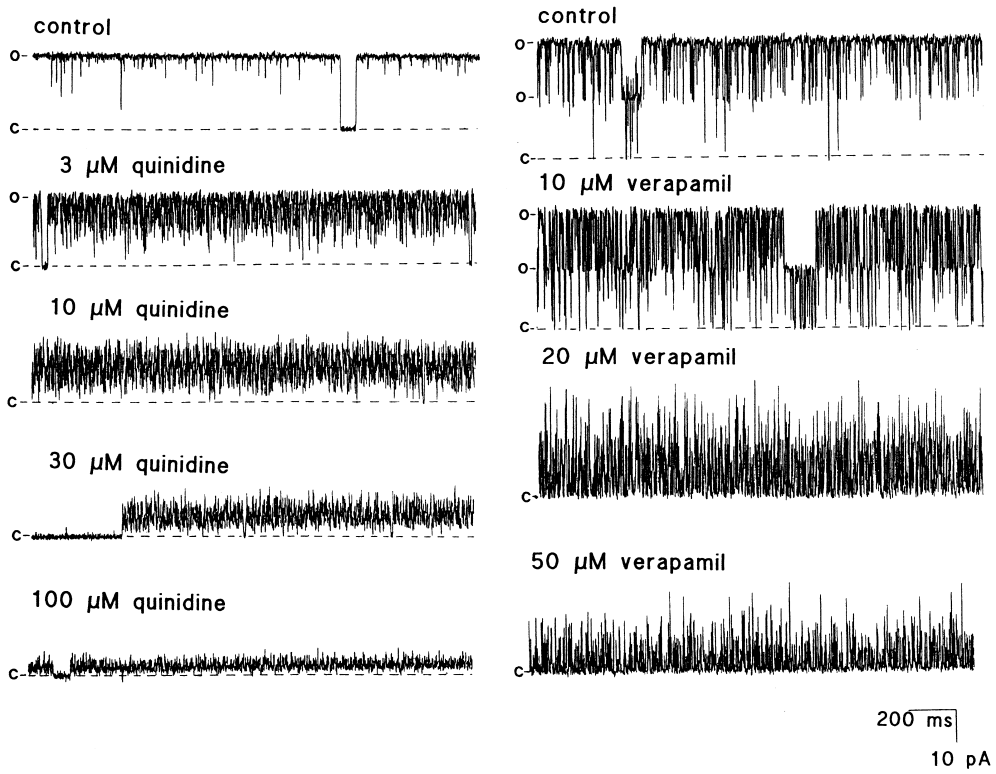

Fig. 3. Examples of the concentration-dependent effects of quinidine and verapamil, added to the *cis* and *trans* solutions, on colonic BK_{Ca} channel activity in comparison to controls without these agents; $V_m = +29.8$ (quinidine) and +20.1 mV (verapamil). Symbols and conditions as in Fig. 1. Note that in the verapamil experiment, two BK_{Ca} channels have fused with the bilayer.

(Fig. 4). The inhibition of channel current by *cis* TEA resembles the effect of other cations on this channel which was accounted for by electrostatic screening of negative charges in the channel entrance [7].

The chromanol compound 293B, an inhibitor of the cAMP-activated K_VLQT1 channel, a very small conductance K^+ channel [24], did not alter colonic BK_{Ca} channel activity (Fig. 5). Clotrimazole, a blocker of the SK4 channel, a small Ca^{2+}-activated K^+ channel [25], had a negligible effect on the current amplitude of the BK_{Ca} channel but reduced its open state probability, P_o, at high concentrations (Fig. 5).

The sulphonylurea agent tolbutamide, a specific blocker of K_{ATP} channels [26], did not affect epithelial BK_{Ca} channel activity (Fig. 5).

The mean, concentration-dependent effects of the various K^+ channel blockers on G_c, E_r and P_o of the reconstituted BK_{Ca} channels are summarised in Table 1.

3.2. Macroscopic basolateral K^+ conductance

3.2.1. Properties of the amphotericin-permeabilised epithelium

The characteristics of the basolateral membrane of rabbit colon epithelium were studied in tissues in which the luminal membrane had been functionally removed with the pore-forming antibiotic amphotericin B. Luminal addition of 10 μg/ml amphotericin to rabbit colon epithelium with K^+-gluconate buffer on the luminal side and Na^+-gluconate buffer on the serosal side resulted in an exponential increase in I_{sc} from 37 ± 21 to 378 ± 147 μA/cm^2 ($n = 156$) at 30 min, and G_t was elevated from 3.0 ± 0.9 to 9.8 ± 3.0 mS/cm^2. The high conductance of the colon epithelium in the presence of amphotericin indicates that the resistance of the luminal membrane had been eliminated for the most part, as values of 10.0 and 10.5 mS/cm^2 were reported earlier for the basolateral membrane conductance of this epithelium from microelectrode studies [10,27]. Further, addition of the

related polyene antibiotic nystatin was shown to reduce the voltage–divider ratio (ratio of luminal to basolateral membrane resistance) practically to zero [27].

When the luminal membrane conductance is selectively increased by amphotericin and the electromotive force, e.m.f., of the luminal membrane is reduced close to zero, it follows from equivalent circuit analysis that G_t should be related to I_{sc} according to,

$$G_t = \frac{I_{sc}}{E_{bl}} + G_s \quad (1)$$

[27] where E_{bl} is the effective e.m.f. across the basolateral membrane and G_s the conductance of the paracellular shunt. This equation implies that the

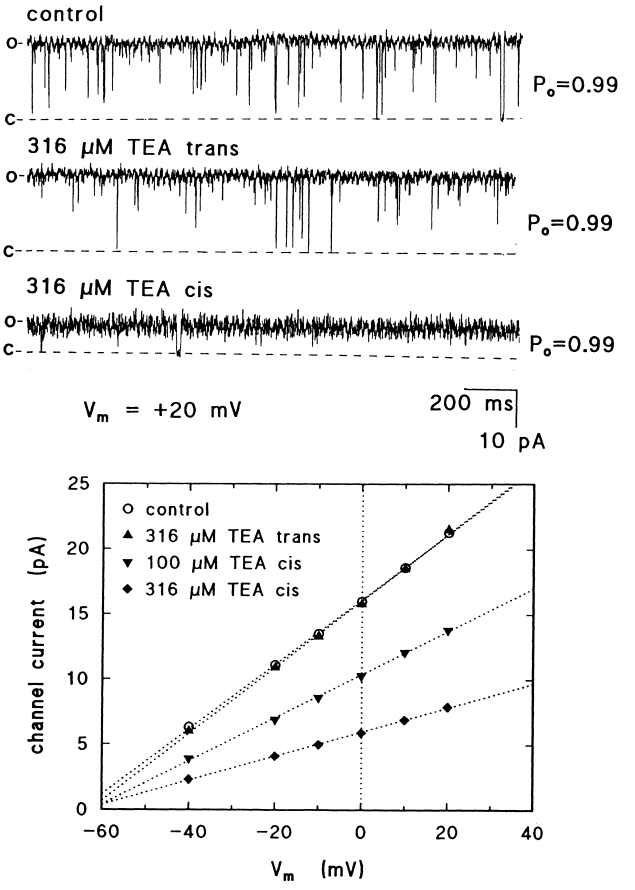

Fig. 4. Example of the effect of TEA added to the *trans* solution and subsequently to the *cis* solution on BK_{Ca} channel activity (top) and the current–voltage relations of this channel in the absence and presence of TEA (bottom). P_o, open-state probability of the channel. Other symbols and conditions as in Figs. 1 and 3.

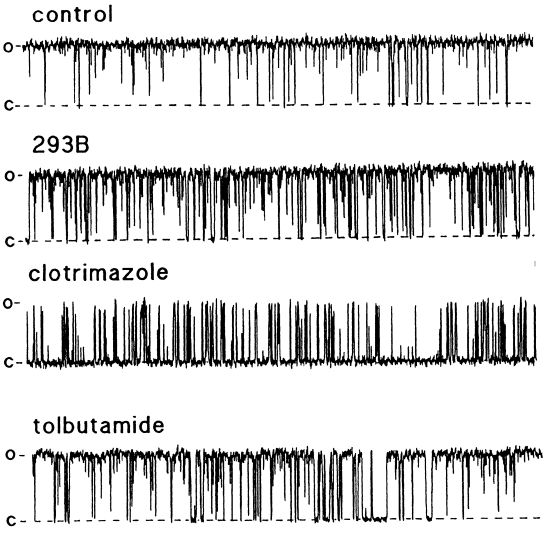

Fig. 5. Examples of the effects of 239B (50 μM), clotrimazole (100 μM) and tolbutamide (200 μM), added to the *cis* and *trans* solutions, on colonic BK_{Ca} channel activity; $V_m = +19.8$ to +20.3 mV. Control, representative channel activity without a channel blocker. Other symbols as in Figs. 1 and 3.

G_t versus I_{sc} relation is linear if E_{bl} and G_s are not altered during the amphotericin permeabilisation of the luminal membrane. As shown in Fig. 6a, linearity of the G_t–I_{sc} relation was indeed observed. It follows from this analysis that E_{bl} is -49.7 ± 4.0 mV ($n = 156$) and G_s 2.0 ± 0.8 mS/cm^2 and that these values are constant after luminal addition of amphotericin. Using the microelectrode technique, the basolateral e.m.f. was estimated to be -53 mV [10] and values of 2.9 and 1.4 mS/cm^2 were reported for G_s based on much smaller numbers of experiments [10,27].

Taken together, these findings indicate that selective permeabilisation of the luminal cell membrane of rabbit colon epithelium with amphotericin B in conjunction with a high-K^+ luminal bathing solution that mimics the intracellular K^+ concentration results in a preparation that has the properties of the basolateral membrane.

Current–voltage (*I–V*) relations of colon epithelia, in which the luminal membrane had been functionally removed by amphotericin, are illustrated in Fig. 6b. With a lumen to serosa K^+ gradient of 145 to 5.4 mM, the *I–V* relation showed outward rectification with a conductance of 11.1 mS/cm^2 between +20 and +40 mV but only 6.0 mS/cm^2 between -120 and -100 mV. When K^+ concentrations were equal on

Table 1
Concentration dependence of the effects of various K^+ channel inhibitors on single-channel conductance, G_c, the reversal potential, E_r, and the open-state probability, P_o, of colonic BK_{Ca} channels reconstituted in planar lipid bilayers

	G_c (pS)	E_r (mV)	P_o	n
Quinidine (μM)				
Control	205 ± 50	−57.0 ± 16.0	0.98 ± 0.01	7
3	162 ± 70	−61.7 ± 9.8	0.98 ± 0.01	4
10	129 ± 57	−62.1 ± 14.0	0.86 ± 0.05	4
30	87 ± 45*	−66.2 ± 21.2	0.77 ± 0.04	3
100	81 ± 63*	−61.6 ± 42.3	0.64 ± 0.36	6
TEA (μM)				
Control	250 ± 22	−62.1 ± 9.0	0.74 ± 0.33	7
10	240 ± 26	−53.9 ± 10.1	0.79 ± 0.12	4
31.6	225 ± 19	−48.2 ± 19.1	0.66 ± 0.23	4
100	147 ± 25*	−60.3 ± 13.9	0.65 ± 0.34	7
316	84 ± 41**	−55.9 ± 24.8	0.54 ± 0.38	4
293B (μM)				
Control	252 ± 32	−61.3 ± 9.7	0.66 ± 0.37	7
10	229 ± 27	−66.6 ± 6.8	0.54 ± 0.45	4
50	269 ± 39	−54.8 ± 6.8	0.86 ± 0.15	5
100	226 ± 52	−64.8 ± 18.1	0.86 ± 0.13	5
Clotrimazole (μM)				
Control	242 ± 38	−62.7 ± 8.7	0.83 ± 0.26	11
10	231 ± 24	−56.7 ± 4.8	0.45 ± 0.27	8
31.6	224 ± 21	−57.3 ± 6.1	0.32 ± 0.23**	9
100	212 ± 22	−65.9 ± 7.3	0.28 ± 0.18**	5
Tolbutamide (μM)				
Control	248 ± 20	−57.6 ± 4.8	0.97 ± 0.02	6
200	239 ± 17	−59.2 ± 5.3	0.93 ± 0.07	6
400	246 ± 10	−62.6 ± 7.3	0.85 ± 0.00	3

P_o of the BK_{Ca} channels is voltage-dependent [13,14]; the P_o value given is that at zero transmembrane voltage. The K^+ channel inhibitors were added at the indicated concentrations to both the *cis* and the *trans* side of the bilayer; the effects are compared to the activity of the same channels before addition of the individual K^+ channel blockers (control). The effects of verapamil are not shown; this agent caused rapid current fluctuations (see Fig. 3) that could not be analysed with the pCLAMP program. *$P < 0.01$ compared to control. **$P < 0.001$ compared to control.

both sides of the tissue, the *I–V* relation was linear, indicating that the non-linearity of the *I–V* relation in the presence of a K^+ concentration gradient is due to Goldman rectification.

Addition of 5 mM Ba^{2+} (as the acetate salt) markedly inhibited the current across the epithelium. In the presence of a lumen to serosa K^+ gradient, the Ba^{2+}-sensitive current (i.e. the difference of current with and without Ba^{2+}) also showed outward rectification (Fig. 6b). The Ba^{2+}-inhibitable current is presumed to reflect K^+ flow across the basolateral membrane. When we fitted the voltage dependence of the Ba^{2+}-sensitive current with the Goldman–Hodgkin–Katz equation for a single permeant cation, a permeability coefficient of the basolateral membrane for K^+, P_K^{bl}, of 2.6×10^{-5} cm/s ($n = 102$) was obtained. In three preparations of rabbit colon epithelium Wills and coworkers [28] measured an average P_K^{bl} of 6.5×10^{-5} cm/s; the corresponding value in monolayers of A6 cells was 4.2×10^{-5} cm/s [9].

It should be noted that 5 mM Ba^{2+} neither reduced I_{sc} nor the reversal potential of the *I–V* relation to zero (Fig. 6b). Inhibition of I_{sc} was a saturable function of Ba^{2+} concentration with a half-saturating Ba^{2+} concentration of 3.8 mM and a maximum inhibitory effect of 448 μA/cm^2 (Fig. 6c). This maximum inhibitory effect of Ba^{2+} was close to the control I_{sc} without Ba^{2+} in these tissues, 498 μA/cm^2, suggesting that infinite Ba^{2+} concentrations block I_{sc} practically completely. Hence we conclude that I_{sc} is carried almost totally by potassium ions under the conditions of our experiments.

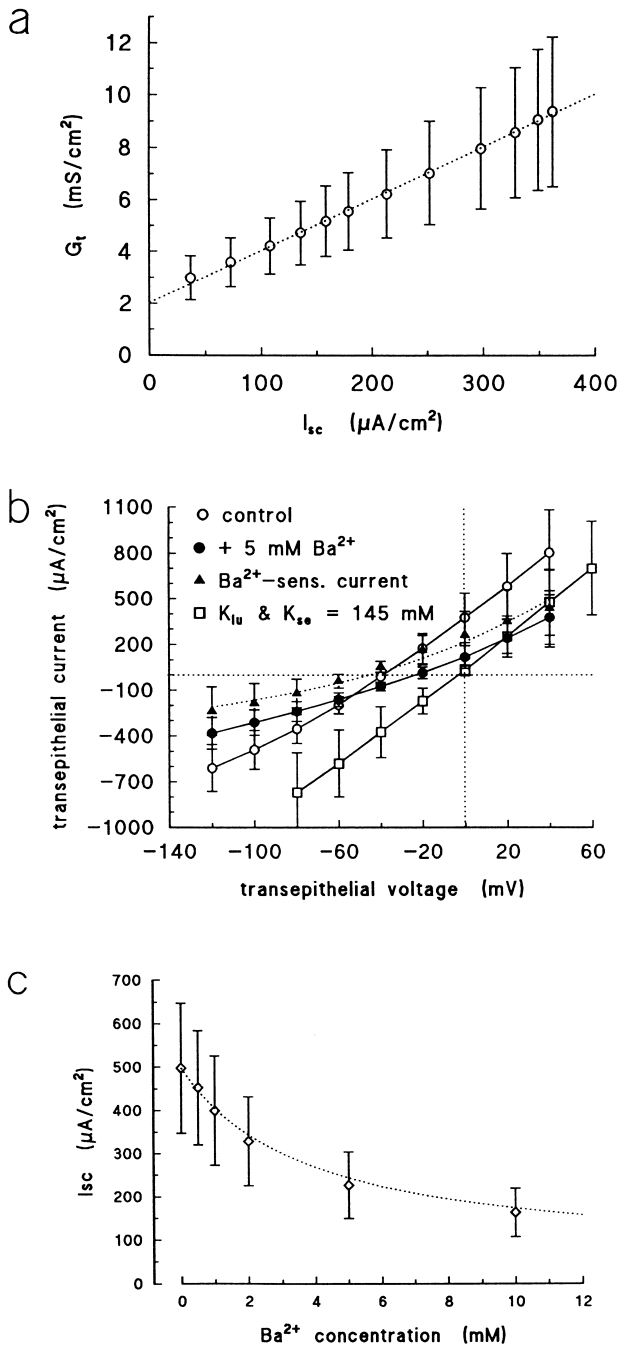

Fig. 6. Amphotericin-permeabilised colon epithelia with a high-K^+ luminal bathing solution. (a) Relation of transepithelial conductance, G_t, and short-circuit current, I_{sc}, following addition of amphotericin B to the luminal bathing solution. Means ± S.D. of 161–171 experiments. The dotted line represents linear regression analysis according to Eq. 1; the correlation coefficient was 0.99. (b) Transepithelial current–voltage (*I–V*) relations with a lumen to serosa K^+ gradient of 145 to 5.4 mM under control conditions and in the presence of 5 mM Ba^{2+} or with 145 mM K^+ on both sides of the tissue without Ba^{2+}. The dotted curve represents the fit of the Ba^{2+}-sensitive *I–V* curve to the Goldman–Hodgkin–Katz equation for a single permeant cation. Means ± S.D. of 101–115 experiments with a K^+ gradient and 14 experiments without a K^+ gradient. (c) Concentration dependence of the inhibitory effect of Ba^{2+} on I_{sc}. The dotted curve was calculated by approximating the parameters of simple saturation kinetics to the measured values using non-linear regression analysis. Means ± S.D. of five experiments.
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3.2.2. *Inhibition of the macroscopic basolateral conductance by K^+ channel blockers*

Amphotericin-permeabilised colon epithelia in the presence of a high-K^+ luminal bathing solution were used to test the effects of various K^+ channel blockers on the macroscopic basolateral K^+ conductance. The properties of amphotericin-permeabilised preparations are stable for approx. 20 min after the peak response of tissue conductance had been reached, hence all experimental procedures on a single tissue were performed within this time frame. To estimate the time-dependent changes in tissue properties, two control *I–V* curves were obtained on every epithelium before addition of K^+ channel blockers with a time interval of 5 min. The difference between these initial *I–V* curves will be termed '5 min control' and is shown in each of the figures that illustrate the effects of K^+ channel blockers on the basolateral *I–V* relations (Fig. 7–10). These small unspecific time-dependent changes should be related to the effects of the individual K^+ channel blockers, especially when the latter are small. Finally, 5 mM Ba^{2+} was added to both the luminal and serosal bathing solution. The current inhibited by the individual K^+ channel blockers was compared to the current inhibited by Ba^{2+} in the same tissues.

The current across the basolateral membrane was inhibited by quinidine in a concentration-dependent manner (Fig. 7). Thus, a quinidine-sensitive basolateral conductance is present in rabbit colon epithelium under 'basal' or 'resting' conditions, whereas in turtle colon epithelium and A6 cells a quinidine-inhibitable conductance is observed only under conditions of cell swelling [8,9].

Surprisingly, quinidine was a more potent blocker of basolateral current when added to the lumen side of the epithelium than to the serosa side (Fig. 7). Apparently, quinidine gains access to the basolateral

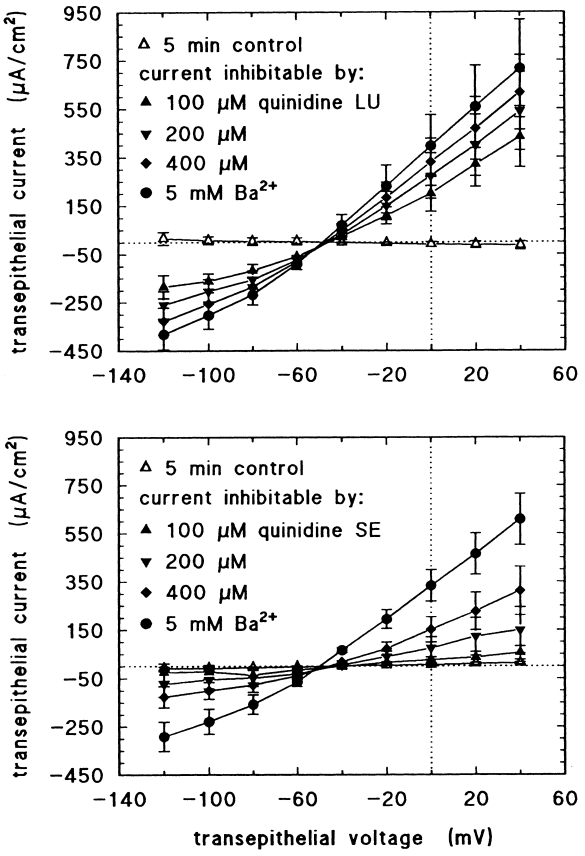

Fig. 7. Effect of quinidine on the transepithelial current–voltage (*I–V*) relation of amphotericin-permeabilised rabbit colon epithelia with 145 K^+ on the luminal side of the tissues and 5.4 mM K^+ on the serosal side. Shown is the current inhibited by increasing concentrations of quinidine added in 5 min intervals to the luminal solution (top) or the serosal solution (bottom). For comparison, the current inhibited by 5 mM Ba^{2+}, added to both the luminal and the serosal side of the same tissues at the conclusion of these experiments, and the unspecific change of the *I–V* curves with time ('5 min control', i.e. the difference between two *I–V* curves obtained with a time interval of 5 min before addition of a K^+ channel blocker) are also given. Means ± S.E.M. of 5–6 experiments.

K^+ channels more easily from the luminal solution than from the serosal solution. In an earlier study on turtle colon epithelium, quinidine was shown to inhibit basolateral K^+ conductance with a slower time course when added to the serosal bath than after luminal addition. This phenomenon was attributed to the large unstirred connective tissue layer on the serosal side of the epithelium [8].

The side dependence of the inhibitory effect of K^+ channel blockers on basolateral current was especially pronounced with TEA which decreased current and conductance only when added to the luminal bathing solution; from the serosal side TEA was ineffective (Fig. 8). The luminal TEA concentrations necessary to reduce the basolateral current were much higher than those required for inhibition of single BK_{Ca} channels in the lipid bilayer system (see Fig. 4).

Verapamil, added to both the lumen and the serosa side of the tissue, was as strong an inhibitor of macroscopic basolateral conductance as quinidine and TEA but at lower concentrations (Fig. 9a). At 100 μM, verapamil decreased transepithelial K^+ current almost as potently as 5 mM Ba^{2+}.

The chromanol 293B, which did not affect single BK_{Ca} channel activity, had no or only a very small inhibitory effect on basolateral conductance (Fig. 9b). As will be shown below, 293B abolished Cl^- secretion induced by 5′-(*N*-ethylcarboxamido)adeno-

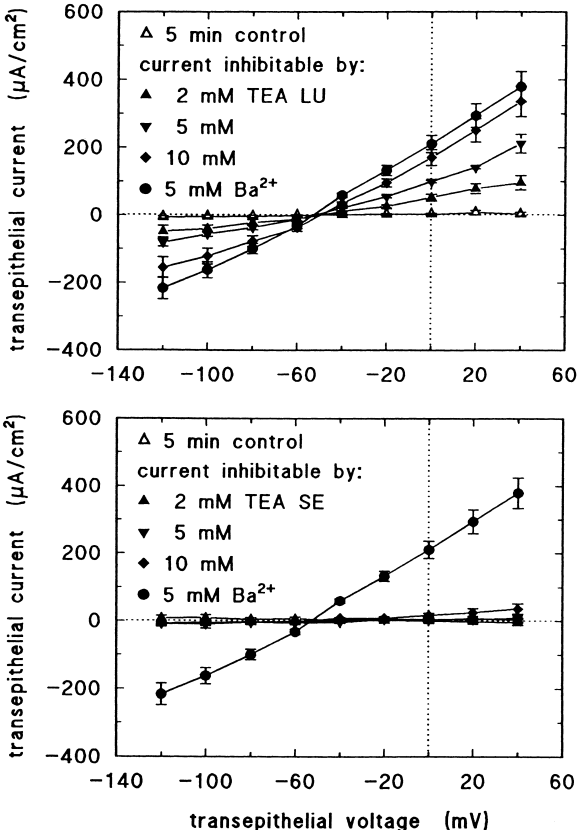

Fig. 8. Effect of TEA on the transepithelial *I–V* relation. Shown is the current inhibited by increasing concentrations of TEA added to the luminal solution (top) or the serosal solution (bottom). Other conditions as in Fig. 7. Means ± S.E.M. of 6–8 experiments.

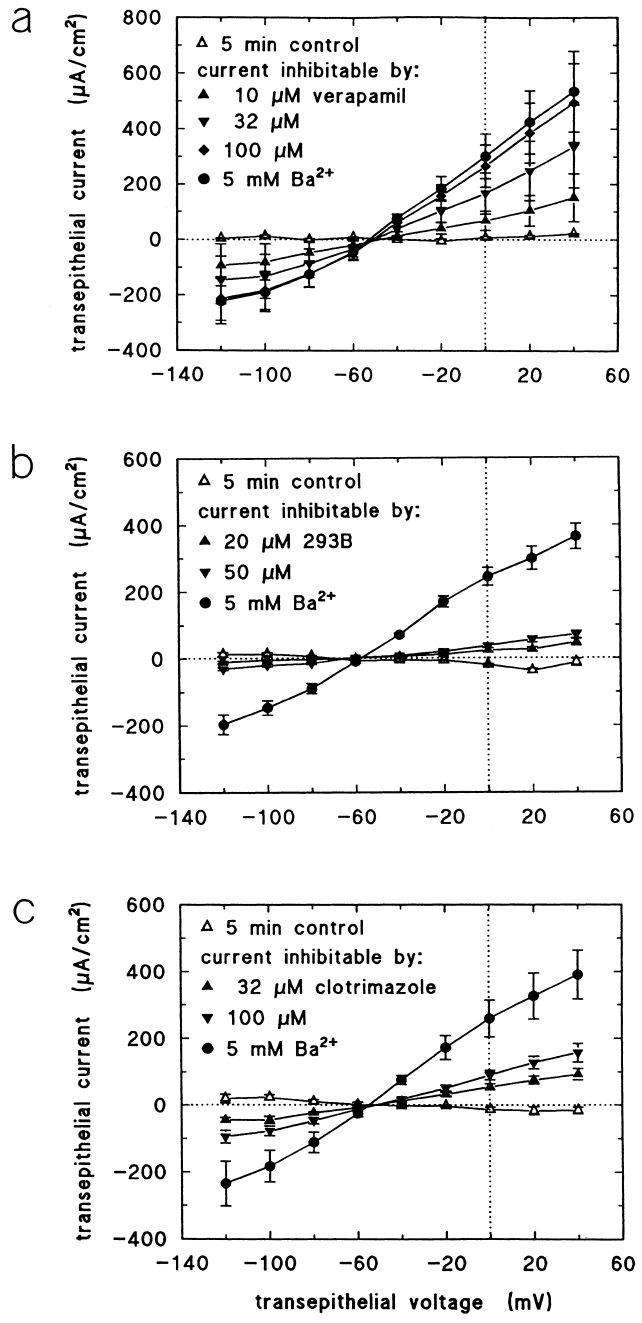

Fig. 9. Effects of verapamil (a), 293B (b), and clotrimazole (c) on the transepithelial *I–V* relation. Shown is the current inhibited by increasing concentrations of the individual agents added to both sides of colon epithelia. Other conditions as in Fig. 7. Means ± S.E.M. of 5–7 experiments.

sine (NECA). It is possible that basolateral K^+ conductance is increased in states of Cl^- secretion and that this increase is inhibited by 293B. But serosal addition of NECA to amphotericin-permeabilised epithelia only caused a small increase in basolateral K^+ conductance and the inhibitory effect of 293B was not larger in NECA-stimulated tissues than in control tissues (data not shown).

The inhibition of basolateral conductance produced by clotrimazole was stronger than that of 293B but weaker than that of quinidine and verapamil (Fig. 9c).

ChTX, which is a very potent blocker of reconstituted BK_{Ca} channels from rabbit colon epithelium [14], had only a weak effect on basolateral conductance (Fig. 10a). In the ChTX experiments the epithelia were pretreated with 50 μg/ml bovine serum albumin to prevent unspecific tissue binding of ChTX.

The inhibition of basolateral K^+ current caused by tolbutamide, at very high concentrations, was ap-

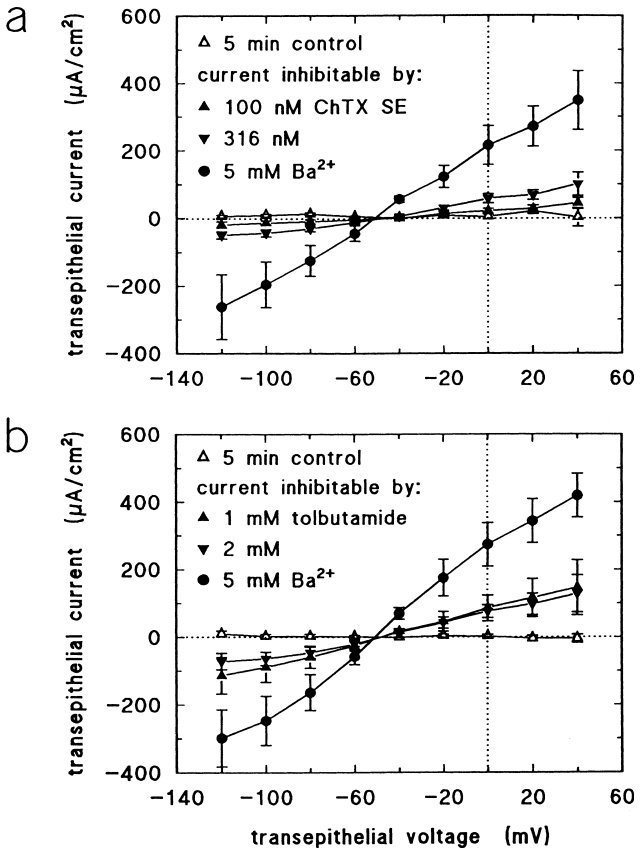

Fig. 10. Effects of ChTX (a) and tolbutamide (b) on the transepithelial *I–V* relation. ChTX was added to the serosal side of the tissue, tolbutamide to both sides. Shown is the current inhibited by increasing concentrations of the individual agents. Other conditions as in Fig. 7. Means ± S.E.M. of 5–8 experiments.

proximately equivalent to that produced by clotrimazole (Fig. 10b), but compared to the marked reduction of basolateral K^+ current caused by tolbutamide in A6 cells [26] and *Necturus* small intestine [29], the inhibitory effect of this agent on basolateral K^+ conductance of rabbit colon epithelium was small.

Possibly, the cellular ATP levels are high under our experimental conditions, resulting in closure of the K_{ATP} channels and weak inhibition of basolateral K^+ current by tolbutamide. We therefore also tested the effect of tolbutamide on epithelia in which ATP was decreased by replacing D-glucose with mannitol in the bathing solutions. Mannitol is no substrate for the ATP-producing cell metabolism. Indeed, substitution of mannitol for glucose resulted in a large exponential increase in tissue conductance of amphotericin-permeabilised epithelia, but only a small fraction of the mannitol-induced conductance was inhibited by tolbutamide (data not shown). Hence ATP-inhibitable K^+ channels seem to be present in the basolateral membrane of rabbit colonocytes, but the sensitivity of these channels to tolbutamide is low.

3.3. Effects of K^+ channel blockers on transepithelial Na^+ absorption and Cl^- secretion

The functional involvement of different classes of K^+ channels in transepithelial Na^+ and Cl^- transport was examined using various K^+ channel blockers in intact colon epithelia mounted in Ussing chambers with physiological NaCl Ringer solution on both sides of the tissue. 1 μM tetrodotoxin was present in the serosal solution in both the Na^+ absorption and Cl^- secretion experiments to exclude neuronal effects of the tested K^+ channel blockers on electrolyte transport. Tetrodotoxin was shown to abolish neuronally mediated Cl^- secretion but to have no direct effect on colonocytes [30].

Na^+ absorption was measured in epithelia from rabbits on a low Na^+ diet to increase endogenous aldosterone secretion and amiloride-sensitive Na^+ transport. Under these conditions, the short-circuit current, I_{sc}, is equal to net Na^+ transport [12].

The average effects of the K^+ channel blockers tested on transepithelial Na^+ transport are summarised in Table 2. In contrast to amiloride, 5 mM Ba^{2+} inhibited I_{sc} only partially. Other than Ba^{2+}, quinidine and verapamil were the only K^+ channel blockers that reduced Na^+ transport markedly. TEA, 293B, tolbutamide, and ChTX did not affect Na^+ transport in concentrations higher than those known to block various types of K^+ channels. Clotrimazole inhibited Na^+ absorption somewhat, but this effect was not statistically significant.

In other epithelia Cl^- secretion was stimulated by serosal addition of 0.4 mM NECA, an adenosine derivative that acts via the cAMP pathway [11]. Cl^- secretion was studied in epithelia in which Na^+ transport had been abolished with 100 μM amiloride in the luminal solution. Under these conditions, the rise in I_{sc} caused by NECA is equivalent to the rate of Cl^- secretion.

Of the tested K^+ channel blockers, Ba^{2+} and 293B abolished Cl^- secretion completely, and quinidine

Table 2
Effects of various K^+ channel inhibitors, added to both the luminal and the serosal bathing solution, on transepithelial Na^+ absorption in rabbit colon epithelium

	I_{sc} (μA/cm^2)			n
	Control	K^+ channel inhibitor	Amiloride	
Ba^{2+} (5 mM)	94.1 ± 9.4	46.4 ± 6.0**	−2.2 ± 1.7	24
Quinidine (0.8 mM)	94.9 ± 11.3	30.1 ± 4.4**	−3.5 ± 1.4	18
Verapamil (100 μM)	98.4 ± 11.3	62.0 ± 5.8*	0.2 ± 2.0	16
TEA (10 mM)	87.8 ± 12.0	80.0 ± 11.6	5.8 ± 1.9	12
293B (50 μM)	83.7 ± 14.5	73.1 ± 17.1	−2.9 ± 5.4	7
Clotrimazole (100 μM)	75.6 ± 9.5	59.0 ± 8.7	6.3 ± 3.6	7
Tolbutamide (1 mM)	87.7 ± 11.7	73.7 ± 10.7	−4.7 ± 2.4	18
ChTX (230 nM)	105.1 ± 39.3	103.4 ± 40.2	6.4 ± 8.2	4

The rate of Na^+ absorption is set equal to the short-circuit current, I_{sc}. The effects of the individual K^+ channel inhibitors were compared to that of 100 μM amiloride added subsequently to the same tissues. Means ± S.E.M. *$P < 0.01$ compared to control. **$P < 0.001$ compared to control.

Table 3
Effects of pretreatment with various K^+ channel inhibitors, added to both the luminal and the serosal bathing solution, on transepithelial Cl^- secretion in rabbit colon epithelium elicited by NECA, 0.4 mM in the serosal solution

	Increase in I_{sc} ($\mu A/cm^2$) caused by NECA		n
	Control	K^+ channel inhibitor	
Ba^{2+} (5 mM)	56.5 ± 5.3	5.2 ± 4.1*	9
Quinidine (0.8 mM)	78.4 ± 9.1	29.8 ± 5.1*	10
Verapamil (100 μM)	73.8 ± 4.3	74.3 ± 9.7	5
TEA (5 mM)	56.5 ± 8.3	60.9 ± 2.0	4
293B (50 μM)	68.0 ± 8.6	1.6 ± 4.1*	6
Clotrimazole (100 μM)	52.6 ± 6.4	35.9 ± 7.8	7
Tolbutamide (1 mM)	72.1 ± 14.3	56.4 ± 7.2	6
ChTX (230 nM)	63.4 ± 10.5	59.8 ± 8.1	5

Na^+ absorption had been abolished with 100 μM amiloride in the luminal solution. Cl^- secretion is set equal to the increase in I_{sc} caused by NECA. Control: Cl^- secretion in paired tissues without a K^+ channel inhibitor. Means ± S.E.M. *$P < 0.001$ compared to control.

was a partial inhibitor (Table 3). Reduction of Cl^- secretion by Ba^{2+} has been shown earlier in various epithelia [3,31,32]. Verapamil that decreased Na^+ transport did not change Cl^- secretion. The inhibitory effect of clotrimazole on Cl^- secretion was variable; on average it did not reach statistical significance. TEA and ChTX, blockers of BK_{Ca} channels, did not affect Cl^- secretion nor did the K_{ATP} channel inhibitor tolbutamide.

4. Discussion

Three experimental approaches were used to assess the presence and functional role of various classes of K^+ channels in the basolateral membrane of rabbit colon epithelium: single-channel measurements in the lipid bilayer reconstitution system, recordings of the macroscopic basolateral K^+ conductance in amphotericin-permeabilised epithelia, and finally monitoring the effects of more or less selective K^+ channel blockers on transepithelial Na^+ absorption and Cl^- secretion.

The predominant K^+ channel type in the basolateral membrane was the high-conductance, Ca^{2+}-activated and voltage-gated K^+ channel. Similarly, iberiotoxin, a selective blocker of BK_{Ca} channels, was found to inhibit 75% of the Ba^{2+}-sensitive Rb^+ uptake in basolateral membrane vesicles from rabbit colonocytes, indicating that BK_{Ca} channels are the most abundant K^+ channels in the basolateral membrane of colon epithelium [33].

Low-conductance K^+ channels were also seen in the present reconstitution experiments but much less frequently. The small K^+ channels showed channel rundown, possibly because a cytosolic component is required to sustain channel activity. Hence it was not possible to study the inhibitory effects of K^+ channel blockers on the small K^+ channel reliably.

Macroscopic basolateral K^+ conductance, that comprises the conductances of the basolateral membranes of many cells present in the epithelium, was inhibited by Ba^{2+}, quinidine, verapamil and TEA, whereas ChTX, 239B, clotrimazole and tolbutamide were weak inhibitors. Quinidine and verapamil were also reported to inhibit Rb^+ uptake into basolateral membrane vesicles of rabbit colonocytes as potently as Ba^{2+} while ChTX and sulphonylurea agents had no or only a small inhibitory effect on Rb^+ uptake in this vesicle preparation [34].

4.1. Basolateral K^+ channels involved in Na^+ absorption

Ba^{2+}, quinidine and verapamil, agents that inhibit both single BK_{Ca} channel activity and macroscopic basolateral K^+ conductance, also decrease transepithelial Na^+ absorption. The agreement of these findings suggests that the Ba^{2+}-, quinidine- and verapamil-sensitive K^+ channels that seem to be involved in basolateral K^+ recycling during active Na^+ transport in rabbit colon epithelium are the BK_{Ca} channels.

But TEA that also inhibits both BK_{Ca} channels and basolateral K^+ conductance, and ChTX that

blocks single BK_{Ca} channels without influencing basolateral K^+ conductance, do not inhibit Na^+ absorption. TEA decreased basolateral K^+ current in amphotericin-permeabilised epithelia only when added to the luminal solution; from the serosal solution TEA was ineffective. In single-channel experiments, TEA also inhibited BK_{Ca} channels only from the side from which K^+ flow originates. Hence it appears that the small polar TEA molecules enter the cells through the amphotericin pores and inhibit basolateral K^+ flux from the inside of the cells.[3] Without amphotericin TEA cannot enter cells and therefore does not inhibit basolateral K^+ outflow.

ChTX, on the other hand, is thought to block BK_{Ca} channels only from the extracellular side. But internal K^+ relieves channel inhibition by external ChTX, rendering ChTX ineffective at high intracellular K^+ concentrations as shown for BK_{Ca} channels from rat skeletal muscle [36], canine smooth muscle [37] and rabbit colon epithelium [14]. Apparently ChTX is 'knocked off' its external blocking site by K^+ outflow from the cell interior, an effect termed 'trans-enhanced dissociation' [36].

According to this evidence, serosal addition of TEA and ChTX cannot be expected to reduce Na^+ absorption in intact epithelia. Taken together, the present results are consistent with the notion that BK_{Ca} channels are involved in basolateral K^+ recycling that is necessary to maintain Na^+ absorption in colon epithelium, but basolateral K^+ conductance has to be reduced markedly for inhibition of Na^+ transport to occur, as the decrease of P_o of BK_{Ca} channels caused by clotrimazole was insufficient to reduce Na^+ transport significantly.

A role for BK_{Ca} channels in Na^+ absorption is also supported by the 10-fold higher abundance of this channel type in rabbit colon surface cells which are considered to be responsible for active Na^+ transport than in crypt cells that are presumed to account for Cl^- secretion [33].

Can it be excluded that basolateral K^+ channels other than the BK_{Ca} channel participate in active transepithelial Na^+ transport? Quinidine was reported to block not only BK_{Ca} channels but also a low-conductance K^+ channel in the basolateral membrane of epithelial cells [23,38] and the K^+ channel induced by cell swelling [8,9]. Verapamil inhibits delayed rectifier K^+ channels [17–19] aside from smooth muscle [20] and epithelial BK_{Ca} channels. However, if a small K^+ channel contributes to basolateral K^+ recycling during Na^+ absorption in rabbit colon, this channel does not seem to be the K_VLQT1 or SK4 channel as neither 293B nor clotrimazole influenced Na^+ transport significantly.

Possibly, the type of K^+ channel that is responsible for basolateral K^+ recycling during amiloride-sensitive Na^+ absorption differs among epithelia and species. Aldosterone, which is required for expression of amiloride-sensitive Na^+ absorption in rabbit colon epithelium [12], was shown to increase the number of a 15 pS K^+ channel present in the basolateral membrane of *Xenopus laevis* alveolar epithelium [39]. In frog skin, aldosterone was reported to activate tolbutamide-sensitive K^+ channels, and tolbutamide abolished the aldosterone-induced increase in active Na^+ transport in this tissue, albeit with a slow time course [40].

Interestingly, 5 mM Ba^{2+} inhibited transepithelial Na^+ transport by only 50% (see Table 2). Incomplete inhibition of Na^+ transport by Ba^{2+} has also been observed in *Necturus* urinary bladder [41] and dog tracheal epithelium [31]. Reduction of basolateral K^+ current in rabbit colon epithelium was shown in the present experiments to be a hyperbolic function of Ba^{2+} concentration with a half-saturation constant of 3.8 mM. Hence it could be argued that 5 mM Ba^{2+} does not block basolateral K^+ conductance completely. However, 5 mM Ba^{2+} abolished Cl^- secretion totally (see Table 3). Notwithstanding incomplete inhibition of the basolateral K^+ conductance by Ba^{2+}, the possibility has to be considered that part of the K^+ recirculation across the basolateral membrane necessary for Na^+ absorption is mediated by a non-conductive mechanism. Ba^{2+}-in-

[3] The amphotericin pores permit the passage of urea (molecular mass, m.m., 60 Da) with a reflection coefficient of 0.57, whereas glucose (m.m. 180 Da) has a reflection coefficient of 1 and is therefore excluded from these pores [35]. TEA (m.m. 130 Da) is in size between urea and glucose, hence its diffusion through the amphotericin pores most likely is restricted, resulting in intracellular TEA concentrations lower than the extracellular concentrations. This phenomenon may account for the finding that TEA inhibited single BK_{Ca} channels at a concentration of 0.3 mM, whereas 5–10 mM TEA was necessary to decrease macroscopic basolateral K^+ conductance in amphotericin-permeabilised epithelia.

sensitive K^+ efflux has been observed in renal tubules, suggesting the presence of an electroneutral KCl exit mechanism in the basolateral membrane (see [42,43]). In membrane vesicles of rabbit colon epithelium, Ba^{2+} was shown to inhibit Rb^+ influx by only 40% although uptake via the Na^+/K^+-exchange pump and the $Na^+,2Cl^-,K^+$-cotransport mechanism had been prevented with ouabain and furosemide, respectively [33]. Other evidence for electroneutral basolateral K^+ efflux in rabbit colonocytes has been presented earlier [32].

Similar to Ba^{2+}, quinidine and verapamil also inhibited Na^+ absorption only partially (see Table 2).

4.2. Basolateral K^+ channels involved in Cl^- secretion

Transepithelial Cl^- secretion in rabbit colon epithelium was inhibited completely by the chromanol 293B, a selective blocker of the small (<3 pS) K_VLQT1 channel [24], and by Ba^{2+} (see Table 3). Quinidine was a partial inhibitor. The decrease in Cl^- secretion caused by clotrimazole, which impedes the SK4 channel and at high concentrations also the K_VLQT1 channel [25], was variable and did not reach statistical significance. TEA, ChTX, and tolbutamide did not affect Cl^- secretion. These findings suggest that the K_VLQT1 channel is responsible for basolateral K^+ recycling during Cl^- secretion in rabbit colon epithelium. Similarly, 293B inhibited Cl^- secretion stimulated by PGE_2 and 3-isobutyl-1-methylxanthine (IBMX) in rat colon epithelium completely [24].

The resolution of our lipid bilayer reconstitution setup was insufficient to detect a channel of 3 pS or smaller.

The K^+ conductance that is involved in Cl^- secretion contributes little to the total basolateral K^+ conductance, suggesting that the number of Cl^- secreting cells is small compared to the total number of cells present in the epithelium.

293B did not affect Na^+ absorption, whereas verapamil that inhibited Na^+ absorption did not change Cl^- secretion. Hence, different classes of K^+ channels appear to be responsible for basolateral K^+ recycling during Cl^- secretion and Na^+ absorption. In contrast to Na^+ absorption, the basolateral K^+ efflux that participates in Cl^- secretion seems to be totally channel-mediated.

The function of the 31 pS channel observed in the basolateral membrane of rabbit colon epithelium is unclear at present. In two experiments each, 293B and tolbutamide did not affect this channel (data not shown).

Again, in different epithelia different classes of K^+ channels appear to be involved in basolateral recirculation of K^+ during Cl^- secretion. In exocrine glands, BK_{Ca} channels are thought to be responsible for basolateral K^+ exit during fluid and electrolyte secretion [44]. BK_{Ca} channels but not 293B-sensitive K^+ channels also appear to mediate basolateral K^+ efflux during secretion in human colonocytes [45,46]. On the other hand, low-conductance basolateral K^+ channels sensitive to clotrimazole [47] or nicardipine [48] were reported to inhibit Cl^- secretion in T84 cells or rat colon. In rat and rabbit colon epithelium both clotrimazol and 293B have to be present to inhibit carbachol-stimulated Cl^- secretion [25], and in mouse colon mucosa both 293B and ChTX were partial inhibitors of Cl^- secretion [49]. These findings suggest that more than one type of basolateral K^+ channel or a combination of K^+ channels may play a role in maintaining Cl^- secretion in different epithelia.

References

[1] S.G. Schultz, R.L. Hudson, Biology of sodium-absorbing cells: dawning of a new area, in: M. Field, R.A. Frizzell (Eds.), Handbook of Physiology, Sect. 6, The Gastrointestinal System, Vol. IV, Intestinal Absorption and Secretion, Am. Physiol. Soc., Bethesda, MD, 1991, pp. 45–81.

[2] D.R. Halm, R.A. Frizzell, Ion transport across the large intestine, in: M. Field, R.A. Frizzell (Eds.), Handbook of Physiology, Sect. 6, The Gastrointestinal System, Vol. IV, Intestinal Absorption and Secretion, Am. Physiol. Soc., Bethesda, MD, 1991, pp. 257–273.

[3] D.C. Dawson, N.W. Richards, Basolateral K conductance: role in regulation of NaCl absorption and secretion, Am. J. Physiol. 259 (1990) C181–C195.

[4] C.U. Cotton, Basolateral potassium channels and epithelial ion transport, Am. J. Respir. Cell. Mol. Biol. 23 (2000) 270–272.

[5] D.C. Eaton, Y. Marunaka, B.N. Ling, Ion channels in epithelial tissue: single-channel properties, in: J.A. Schafer, H.H. Ussing, P. Kristensen, G. Giebisch (Eds.), Membrane Transport in Biology, Vol. 5, Springer-Verlag, Berlin, 1992, pp. 73–165.

[6] H. Wiener, K. Turnheim, C.H. van Os, Rabbit distal colon

epithelium. I. Isolation and characterisation of basolateral plasma membrane vesicles from surface and crypt cells, J. Membr. Biol. 100 (1989) 147–162.
[7] C. Wachter, K. Turnheim, Inhibition of high-conductance, calcium activated potassium channels of rabbit colon epithelium by magnesium, J. Membr. Biol. 150 (1996) 275–282.
[8] W.J. Germann, M.E. Lowy, S.A. Ernst, D.C. Dawson, Differentiation of two distinct K conductances in the basolateral membrane of turtle colon, J. Gen. Physiol. 88 (1986) 237–251.
[9] M.-C. Broillet, J.-D. Horisberger, Basolateral membrane potassium conductance of A6 cells, J. Membr. Biol. 124 (1991) 1–12.
[10] S.G. Schultz, R.A. Frizzell, H.N. Nellans, Active sodium transport and the electrophysiology of rabbit colon, J. Membr. Biol. 33 (1977) 351–384.
[11] M. Grasl, K. Turnheim, Stimulation of electrolyte secretion in rabbit colon by adenosine, J. Physiol. 346 (1984) 93–110.
[12] K. Turnheim, H. Plass, M. Grasl, P. Krivanek, H. Wiener, Sodium absorption and potassium secretion in rabbit colon during sodium deficiency, Am. J. Physiol. 250 (1986) F235–F245.
[13] K. Turnheim, J. Costantin, S. Chan, S.G. Schultz, Reconstitution of a calcium-activated potassium channel in basolateral membranes of rabbit colonocytes into planar lipid bilayers, J. Membr. Biol. 112 (1989) 247–254.
[14] D.A. Klaerke, H. Wiener, T. Zeuthen, P.L. Jørgensen, Ca^{2+} activation and pH dependence of a maxi K^+ channel from rabbit distal colon epithelium, J. Membr. Biol. 136 (1993) 9–21.
[15] B. Hille, Ion Channels in Excitable Membranes, 2nd edn., Sinauer Associates, Sunderland, MA, 1992.
[16] N. Iwatsuki, O.H. Petersen, Inhibition of Ca^{2+}-activated K^+ channels in pancreatic acinar cells by Ba^{2+}, Ca^{2+}, quinine and quinidine, Biochim. Biophys. Acta 819 (1985) 249–257.
[17] T.W. De Coursey, Mechanism of K^+ channel block by verapamil and related compounds in rat alveolar epithelial cells, J. Gen. Physiol. 106 (1995) 745–779.
[18] D. Chouabe, M.D. Drici, G. Romey, J. Barhanin, M. Lazdunski, HERG and K_vLQT1/I_{sK}, the cardiac K^+ channels involved in long QT syndromes, are targets for calcium channel blockers, Mol. Pharmacol. 54 (1998) 695–703.
[19] R.C. Hogg, C. Trequattrini, L. Catacuzzeno, A. Petris, F. Franciolini, D.J. Adams, Mechanism of verapamil inhibition of action potential firing in rat intracardiac ganglion neurons, J. Pharmacol. Exp. Ther. 289 (1999) 1502–1508.
[20] A.A. Harper, L. Catacuzzeno, C. Trequattrini, A. Petris, F. Franciolini, Verapamil block of large-conductance Ca-activated K channels in rat aortic myocytes, J. Membr. Biol. 179 (2001) 103–111.
[21] R. MacKinnon, R. Latorre, C. Miller, Role of surface electrostatics in the operation of a high-conductance Ca^{2+}-activated K^+ channel, Biochemistry 28 (1989) 8092–8099.
[22] D.I. Cook, E.A. Wegman, T. Ishikawa, P. Poronnik, D.G. Allen, J.A. Young, Tetraethylammonium blocks muscarinically evoked secretion in sheep parotid gland by a mechanism additional to its blockade of BK channels, Pflug. Arch. 420 (1992) 167–171.
[23] R.B. Lomax, G. Warhurst, G.L. Sandle, Characteristics of two basolateral potassium channel populations in human colonic crypts, Gut 38 (1996) 243–247.
[24] K. Kunzelmann, M. Hübner, R. Schreiber, R. Levy-Holzman, H. Garty, M. Bleich, R. Warth, M. Slavik, T. von Hahn, R. Greger, Cloning and function of the rat colonic epithelial K^+ channel K_VLQT1, J. Membr. Biol. 179 (2001) 155–164.
[25] R. Warth, K. Hamm, M. Bleich, K. Kunzelmann, R. von Hahn, R. Schreiber, E. Ullrich, M. Mengel, N. Trautmann, P. Kindle, A. Schwab, R. Greger, Molecular and functional characterization of the small Ca^{2+}-regulated K^+ channel (rSK4) of colonic crypts, Pflug. Arch. 438 (1999) 437–444.
[26] M.-C. Broillet, J.-D. Horisberger, Tolbutamide-sensitive potassium conductance in the basolateral membrane of A6 cells, J. Membr. Biol. 134 (1993) 181–188.
[27] N.K. Wills, S.A. Lewis, D.C. Eaton, Active and passive properties of rabbit descending colon: a microelectrode and nystatin study, J. Membr. Biol. 45 (1979) 81–108.
[28] N.K. Wills, D.C. Eaton, S.A. Lewis, M.S. Ifshin, Current-voltage relationship of the basolateral membrane of a tight epithelium, Biochim. Biophys. Acta 555 (1979) 519–523.
[29] O. Mayorga-Wark, W.P. Dubinsky, S.G. Schultz, Reconstitution of a K_{ATP} channel from basolateral membranes of *Necturus* enterocytes, Am. J. Physiol. 269 (1995) C464–C471.
[30] B. Biagi, Y.-Z. Wang, H.J. Cooke, Effects of tetrodotoxin on chloride secretion in rabbit distal colon: tissue and cellular studies, Am. J. Physiol. 258 (1990) G223–G230.
[31] M.J. Welsh, Barium inhibition of basolateral membrane potassium conductance in tracheal epithelium, Am. J. Physiol. 244 (1983) F639–F645.
[32] H. Plass, A. Gridl, K. Turnheim, Absorption and secretion of potassium by rabbit descending colon, Pflug. Arch. 406 (1986) 509–519.
[33] M. Grunnet, H.-G. Knaus, C. Solander, D.A. Klaerke, Quantification and distribution of Ca^{2+}-activated maxi K^+ channels in rabbit distal colon, Am. J. Physiol. 277 (1999) G22–G30.
[34] H. Wiener, D.A. Klaerke, P.L. Jørgensen, Rabbit distal colon epithelium. III. Ca^{2+}-activated K^+ channels in basolateral plasma membrane vesicles of surface and crypt cells, J. Membr. Biol. 117 (1990) 275–283.
[35] R. Holz, A. Finkelstein, The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B, J. Gen. Physiol. 56 (1970) 125–145.
[36] R. MacKinnon, C. Miller, Mechanism of charybdotoxin block of the high-conductance, Ca^{2+}-activated K^+ channel, J. Gen. Physiol. 91 (1988) 335–349.
[37] A. Carl, M.L. Garcia, J.L. Kenyon, K.M. Sanders, Block of Ca^{2+} activated K channels in smooth muscle from canine GI-tract by charybdotoxin, Biophys. J. 57 (1990) 507a.
[38] D. Chang, D.C. Dawson, Digitonin-permeabilized colonic cell layers. Demonstration of calcium-activated basolateral

K^+ and Cl^- conductances, J. Gen. Physiol. 92 (1988) 281–306.
[39] B. Illek, H. Fischer, W. Clauss, Aldosterone regulation of basolateral potassium channels in alveolar epithelium, Am. J. Physiol. 259 (1990) L230–L237.
[40] V. Urbach, E. van Kerkhofe, D. Maguire, B.J. Harvey, Rapid activation of K_{ATP} channels by aldosterone in principle cells of frog skin, J. Physiol. 491 (1996) 111–120.
[41] H.R. Demarest, A.L. Finn, Interaction between basolateral K^+ and apical Na^+ conductance in *Necturus* urinary bladder, J. Gen. Physiol. 89 (1987) 563–580.
[42] D.G. Warnock, J. Eveloff, K-Cl cotransport systems, Kidney Int. 36 (1989) 412–417.
[43] E.J. Holtzman, S. Kumar, C.A. Faaland, F. Warner, P.J. Logue, S.J. Erickson, G. Ricken, J. Waldman, S. Kumar, P.B. Dunham, Cloning, characterisation, and gene organisation of K-Cl cotransporter from pig and human kidney and *C. elegans*, Am. J. Physiol. 275 (1998) F550–F564.
[44] O.H. Petersen, Potassium channels and fluid secretion, News Physiol. Sci. 1 (1986) 92–95.
[45] D.C. Devor, A.K. Singh, R.A. Frizzell, R.J. Bridges, Modulation of Cl^- secretion by benzimidazolones. I. Direct activation of a Ca^{2+}-activated K^+ channel, Am. J. Physiol. 271 (1996) L775–L784.
[46] B. McNamara, D.C. Winter, J.E. Cuffe, G.C. O'Sullivan, B.J. Harvey, Basolateral K^+ channel involvement in forskolin-activated chloride secretion in human colon, J. Physiol. 519 (1999) 251–260.
[47] D.C. Devor, A.K. Singh, A.L. Gerlach, R.A. Frizzell, R.J. Bridges, Inhibition of intestinal Cl^- secretion by clotrimazole: direct effect on basolateral membrane K^+ channels, Am. J. Physiol. 273 (1997) C531–C540.
[48] G.L. Sandle, I. Butterfield, N.B. Higgs, G. Warhurst, Direct inhibitory effect of nicardipine on basolateral K^+ channels in human colonic crypts, Pflug. Arch. 437 (1999) 596–602.
[49] A.W. Cuthbert, M.E. Hickman, P. Thorn, L.J. MacVinish, Activation of Ca^{2+}- and cAMP-sensitive K^+ channels in murine colonic epithelia by 1-ethyl-2-benzimidazolone, Am. J. Physiol. 277 (1999) C111–C120.