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Rabbit Distal Colon Epithelium: III. Ca^{2+}-Activated K^+ Channels in Basolateral Plasma Membrane Vesicles of Surface and Crypt Cells

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Summary. In the mammalian distal colon, the surface epithelium is responsible for electrolyte absorption, while the crypts are the site of secretion. This study examines the properties of electrical potential-driven $^{86}Rb^+$ fluxes through K^+ channels in basolateral membrane vesicles of surface and crypt cells of the rabbit distal colon epithelium. We show that Ba^{2+}-sensitive, Ca^{2+}-activated K^+ channels are present in both surface and crypt cell derived vesicles with half-maximal activation at 5×10^{-7} M free Ca^{2+}. This suggests an important role of cytoplasmic Ca^{2+} in the regulation of the bidirectional ion fluxes in the colon epithelium.

The properties of K^+ channels in the surface cell membrane fraction differ from those of the channels in the crypt cell derived membranes. The peptide toxin apamin inhibits Ca^{2+}-activated K^+ channels exclusively in surface cell vesicles, while charybdotoxin inhibits predominantely in the crypt cell membrane fraction. Titrations with H^+ and tetraethylammonium show that both high- and low-sensitive $^{86}Rb^+$ flux components are present in surface cell vesicles, while the high-sensitive component is absent in the crypt cell membrane fraction. The Ba^{2+}-sensitive, Ca^{2+}-activated K^+ channels can be solubilized in CHAPS and reconstituted into phospholipid vesicles. This is an essential step for further characterization of channel properties and for identification of the channel proteins in purification procedures.

Key Words Ca^{2+}-activated K^+ channels · rabbit distal colon membranes · reconstitution

Introduction

Specialized epithelial cells in the distal nephron and the mammalian colon adjust the rate of external salt excretion and participate in maintaining ion homeostasis of the entire organism (for reviews *see*, e.g., 35, 37). Detailed characterization of the regulation of transepithelial salt transport and the molecular mechanisms of the ion transport processes is therefore of fundamental biological and pathophysiological significance.

In epithelial cells, K^+ channels are important for selective changes of K^+ permeability of basolateral and luminal membranes. Changes of the net electrochemical potential across the membranes will alter the net driving forces for Na,K,Cl-cotransporters or Na^+ channels and the rate of Na^+ entry into the cell cytoplasm. Since the turnover rate of the Na,K pump depends mainly on the cytoplasmic Na^+ activity [22], the opening and closure of both luminal and basolateral K^+ channels may influence the overall rate of transcellular transport. The mammalian colon displays structural and functional heterogeneity. The surface epithelium is involved in aldosterone-stimulated [12, 39] electrolyte and fluid absorption [35], while the epithelial cells in the crypts are responsible for secretory processes [40]. The evaluation of the role of K^+ channels for the regulation of transepithelial ion transport requires the isolation of the transport systems in membrane vesicles and the characterization of the isolated transport proteins to study the regulatory mechanisms modulating their transport properties.

The present work has been concerned with the properties of basolateral K^+ channels in plasma membrane vesicles derived from surface and crypt cells of the rabbit distal colon epithelium [43]. To overcome the problems of measuring fast channel-mediated isotope fluxes into heterogenous populations of membrane vesicles we used the very sensitive methods of electrical potential-driven $^{86}Rb^+$ uptake into membrane vesicles [23]. This allowed us to characterize the different types of K^+ channels in vesicles from the colonic surface and crypt epithelial cells with respect to Ca^{2+}-activation, Ba^{2+}-inhibition, pH activity-profile and sensitivity to a series of inhibitors. Furthermore, procedures were established for solubilization and reconstitution of Ba^{2+}-sensitive K^+ channels into phospholipid vesicles with preservation of their native properties. This is an important step for the identification of the channel

proteins in purification procedures and for further characterization of channel properties.

Materials and Methods

Materials

Trizma (Tris base), MOPS, HEPES, MES, EGTA, Ca^{2+}-ionophore A23187, phenylmethylsulfonyl fluoride, dithiothreitol, furosemide, verapamil, quinidine (SO_4), apamin and soybean phosphatidylcholine (Type II-S) were obtained from Sigma; 4-aminopyridine, TEA(Cl) and ouabain from Merck; CHAPS from Aldrich. Glibenclamide was a gift from Hoechst; stock solutions (1 mM) were made freshly in ethanol. Charybdotoxin was purified to approximately 40% by the method of C. Miller (*personal communication*). The crude venom from the scorpion *Leiurus quinquestriatus Hebraeus* was from Latoxan, Rosans, France. Sephadex G-50 was from Pharmacia. Dowex beads (50 W × 2, 50–100 mesh, H^+ form; Fluka AG, Switzerland) were converted to the Tris form by 24 hr incubation with Tris base (suspension pH ~10) and finally prepared by repetitive washings with distilled water until the suspension pH dropped to 7.2. $^{86}RbCl$ (1–8 mCi/mg) was purchased from Amersham. All conventional chemicals were analytical grade.

Preparation of Vesicles

Basolateral plasma membrane vesicles of surface (BLMS) and crypt (BLMC) cells of rabbit distal colon epithelium were prepared from mucosal scrapings as described earlier [41] and equilibrated in 250 mM sucrose, 50 mM KCl, 1 mM EGTA, 10 mM MOPS-Tris, pH 7.2. Batches of vesicles (app. 15 mg protein/ml) were prepared from several animals and were quick-frozen in liquid nitrogen and kept at −80°C for transport experiments within the following two weeks.

Reconstitution of K^+ Channel Activity

For reconstitution, aliquots of 200 μl containing 400 μg membrane protein were solubilized by adding 20 μl of 0.5 M CHAPS in 250 mM sucrose, 50 mM KCl, 1 mM EGTA, 1 mM dithiothreitol, 10 mM MOPS-Tris, pH 7.2. Soybean phosphatidyl-choline (50 mg/ml), in 200 μl of the same buffer was sonicated to clarity and solubilized by adding 40 μl of 0.5 M CHAPS. The protein and lipid solutions were mixed immediately in a Carlsberg micropipette and applied to a 1 × 30 cm Sephadex G-50 coarse column equilibrated with 250 mM sucrose, 50 mM KCl, 1 mM EGTA, 10 mM MOPS-Tris, pH 7.2 at 20°C. The vesicles were collected in the void volume as described before [23].

Assay of K^+ Channel Activity

The K^+ channel activity in native and reconstituted vesicles was measured as the potential-driven $^{86}Rb^+$ uptake at 20°C that could be blocked by 5 mM $BaCl_2$ [23]. As described earlier [23], Na,K-ATPase activity was blocked by 10 min preincubation of vesicles (0.3 to 0.6 mg protein per ml) in 0.5 mM ouabain, 2 mM $MgCl_2$, 0.1 mM vanadate, 250 mM sucrose, 50 mM KCl, 1 mM EGTA, 10 mM MOPS-Tris, pH 7.2, at 20°C. Furosemide (1 mM) was added to increase the sensitivity of the flux measurements by blocking the Na,K,Cl-cotransport system present in the colon epithelium [42]. In experiments where stimulatory effects of Ca^{2+} were investigated, vesicles were preincubated for 30 min in 3.75 mM EGTA and 10 μM Ca^{2+}-ionophore A23187 in order to deplete the vesicles of endogenous Ca^{2+} [5, 23]. The presence of A23187 in the membrane ensures that the activities of Ca^{2+} are the same inside and outside the membrane vesicles irrespective of their orientation. In studies of pH effects, the presence of Tris ions can be assumed to abolish H^+ gradients [23].

To avoid artifacts in the dose-response measurements [1], aliquots of the vesicles were incubated in the standard uptake medium for 60 min, divided into several portions and further incubated for 10 min in the absence or presence of the inhibitors tested. If the inhibitors only affect the K^+ conductance, they should inhibit tracer uptake when added at zero time together with $^{86}Rb^+$, but they should not affect the amount of radioactivity after the tracer had accumulated in the vesicles.

Protein was determined according to Bradford [3] using bovine serum albumin as a standard.

Analysis of Dose-Effect Relationships

Data sets ($n \geq 12$) were analyzed by unweighted nonlinear regression methods; the most significant fit was calculated on the basis of the variance ratio (F) test [2]. Equation (1) was used

$$f_{(v)} = 1 - \frac{I_{max} \cdot [I]}{I_{50} + [I]} \quad (1)$$

for a single contributing system (where $f_{(v)}$ is the fractional velocity in the presence of an inhibitor $[I]$, I_{max} is the maximal inhibition and I_{50} is the inhibitor concentration required for half-maximal inhibition), and Eq. (2)

$$f_{(v)} = 1 - \frac{I_{max\,1} \cdot [I]}{I_{50\,1} + [I]} - \frac{I_{max\,2} \cdot [I]}{I_{50\,2} + [I]} \quad (2)$$

for two contributing systems.

Abbreviations

BLMS, basolateral membranes of surface epithelial cells; BLMC, basolateral membranes of crypt epithelial cells; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid; MOPS; 3-(N-morpholino)propanesulfonic acid; MES,2-(N-morpholino) ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; CHAPS, 3-((3-chloramidopropyl)diethylammonio)-1-propanesulfonate; EGTA, ethylene glycol bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid; TEA, tetraethylammonium.

Results

Ba^{2+}-sensitive, Ca^{2+}-activated K^+ channels were observed in both the BLMS and the BLMC fraction. Figure 1 shows the effect of altering Ca^{2+} concentrations in the physiological range [7] using EGTA buffered systems. The addition of Ca^{2+} resulted in a

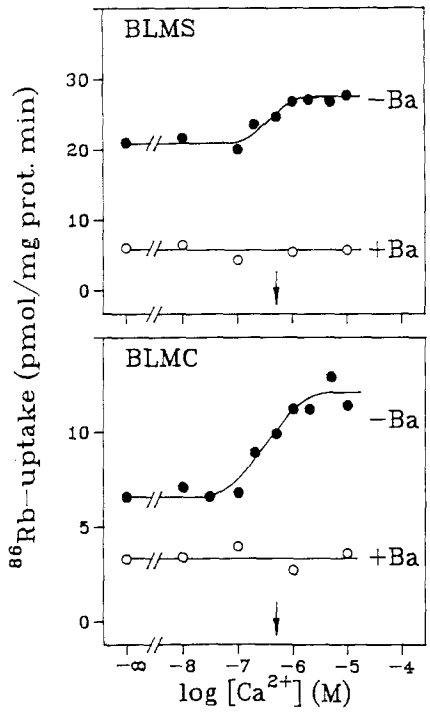

Fig. 1. Ca^{2+}-activation of Ba^{2+}-sensitive $^{86}Rb^+$ flux in basolateral membrane vesicles of rabbit distal colon epithelium. $^{86}Rb^+$ uptakes were performed in the absence (●) and presence (○) of 5 mM $BaCl_2$ at the indicated concentrations of EGTA-buffered free Ca^{2+} (*see* Materials and Methods). *Upper panel:* surface cell (BLMS) fraction; *lower panel:* crypt cell (BLMC) fraction. Half-maximal activatory concentrations are indicated by arrows

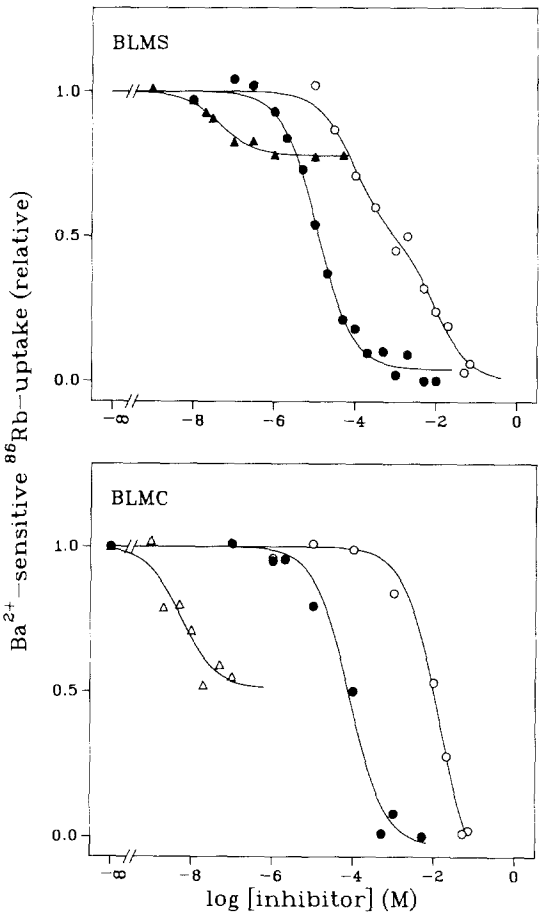

Fig. 2. Dose-response profiles for the inhibition by $BaCl_2$ (●), TEA (○), apamin (▲), and charybdotoxin (△) of the Ba^{2+}-sensitive $^{86}Rb^+$fluxes. Ba^{2+}-sensitive $^{86}Rb^+$uptakes, measured as in Fig. 1 at 10^{-5} M Ca^{2+} and expressed as fractional uptakes as a function of the inhibitor concentration (log scale), are shown with surface (*BLMS*, upper panel) and crypt cell (*BLMC*, lower panel) fractions. Solid curves are computerized best fit for a single (Eq. (1)) or two (Eq. (2)) contributing transport systems (residual sum of squares ≤ 0.06); the inhibition parameters are summarized in the Table

40% (BLMS) and 150% (BLMC) increase of Ba^{2+}-sensitive $^{86}Rb^+$ flux with an apparent half-maximal activation at 0.5 μM free Ca^{2+} for both membrane fractions. The Ba^{2+}-insensitive $^{86}Rb^+$ flux was unaffected by the addition of Ca^{2+}. The specific K^+ channel activity in the presence of 1 μM Ca^{2+} expressed as the Ba^{2+}-sensitive $^{86}Rb^+$ flux into the vesicles was 22 and 8 pmol $^{86}Rb^+$ per mg protein per min for the BLMS and BLMC fractions, respectively.

The fraction of Ba^{2+}-sensitive $^{86}Rb^+$ flux was considerably higher in the BLMS than in the BLMC vesicles (i.e., 71% *versus* 56% inhibition of total $^{86}Rb^+$ flux). The dose-response analysis (Fig. 2) exhibited simple inhibition curves with half-maximal effects (Table) at 12 and 80 μM Ba^{2+} for BLMS and BLMC, respectively.

A variation of pH (Fig. 3) in the range from 8.1 to 5.1 altered Ba^{2+}-sensitive $^{86}Rb^+$ fluxes in both membrane fractions, while background fluxes were unaffected in this pH range. With increasing proton activity, the Ba^{2+}-sensitive $^{86}Rb^+$ fluxes in the BLMC fraction decreased along a sigmoid curve with a pK value of 5.9. In the BLMS fraction the curve was biphasic with apparent half-maximal effects at pK_1 = 7.8 for a high-sensitive flux component and at pK_2 = 5.7 for a low-sensitive component.

Similar distinctions between K^+ channels in surface and crypt vesicles were observed in titrations with TEA (Fig. 2). TEA inhibited $^{86}Rb^+$ fluxes to the same extent as Ba^{2+}. The dose-response curves show only a low sensitivity system in the BLMC fraction (I_{50} = 7500 μM). In the BLMS vesicles two $^{86}Rb^+$ flux components were observed with high (I_{50} = 89 μM) and low (I_{50} = 9400 μM) sensitivities to the inhibitor (Table). The orientation of the vesicles in the membrane fractions used in the present experiments approximates a sealed right-side out: sealed inside-out: leaky ratio of 2 : 1 : 1 [41]. To ex-

Table. Half-maximal inhibitory concentrations (I_{50}) of Ba^{2+}-sensitive $^{86}Rb^+$ fluxes in basolateral membrane vesicles of rabbit distal colon epithelium

Compound	Half-maximal concentration (μM)		
	BLMS		BLMC
	$I_{50\,1}$	$I_{50\,2}$	I_{50}
$BaCl_2$	12		80
$CoCl_2$	400		700
TEA	9400	89	7500
Apamin	0.04		>50
Charybdotoxin	ND		0.005

The values were evaluated by nonlinear regression analysis of the data sets shown in Fig. 2. The symbol (>) indicates that I_{50} is unknown, but is greater than the concentration indicated. ND, not determined; the small fraction of inhibitor-sensitive flux (9%) did not allow the evaluation of I_{50} values with sufficient accuracy.

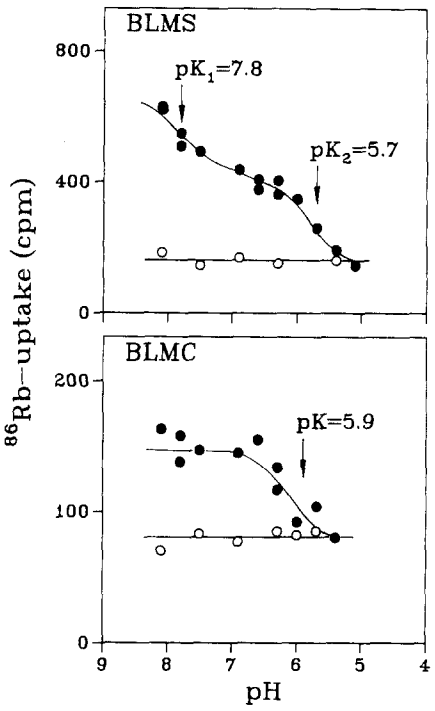

Fig. 3. pH profile of $^{86}Rb^+$ flux in basolateral membrane vesicles of rabbit distal colon epithelium. $^{86}Rb^+$ uptakes were measured as in Fig. 1. at 10^{-5} M Ca^{2+} in the absence (●) or presence (○) of 5 mM $BaCl_2$ in 10 mM solutions of Tris-MOPS (pH 8.1–7.5), MOPS-Tris (pH 7.5–6.3) and MES-Tris (pH 6.3–5.1) overlapping the pH ranges indicated. *Upper panel:* surface cell (*BLMS*) fraction; *lower panel:* crypt cell (*BLMC*) fraction. Note the different uptake scale. The pK values are indicated with arrows

amine if the difference in TEA sensitivity is due to vesicle heterogeneity with respect to orientation, the BLMS vesicles were preloaded for 16 hr with TEA in the same range of concentrations (10 μM to 70 mM) and then used in the $^{86}Rb^+$ uptake. Half-maximal inhibition of $^{86}Rb^+$ flux was observed at 210 μM and 18 mM, respectively, and this is within the range obtained without preloading vesicles with TEA (*see* Table). This is in agreement with the presence of distinct K^+ channels with low and high sensitivity in the BLMS fraction, while only K^+ channels with low sensitivity to TEA were observed in the BLMC fraction.

The bee venom peptide apamin and the scorpion venom peptide charybdotoxin are potent blockers of Ca^{2+}-activated K^+ channels [30] that bind to the extracellular membrane surface [4, 27]. Apamin (50 μM) inhibited 20% of the K^+ channel acivity (Fig. 2) in the BLMS fraction with I_{50} at 40 nM (Table), whereas it was ineffective in the BLMC fraction. Charybdotoxin (0.1 μM), in contrast, inhibited 50% of the K^+ channel activity (Fig. 2) in the BLMC fraction with I_{50} at 5 nM (Table), and showed only a slight inhibition (9%) in the BLMS fraction. The half-maximal inhibitory concentrations for apamin and charybdotoxin are in the same range as values obtained from single channel recordings in other tissues [25, 30].

Suspensions of surface epithelial cells can be isolated by a divalent chelation technique [16] and vesicles can be prepared from these cells as recently described [41]. In this surface cell vesicle preparation, apamin (50 μM) inhibited 54% (n = 3) of the Ba^{2+}-sensitive $^{86}Rb^+$ uptake (180 ± 85 cpm/10 min, n = 3) while charybdotoxin (0.1 μM) had no effect. This demonstrates that the apamin-sensitive K^+ channels are indeed located in the surface epithelial cells and do not originate from nonepithelial elements [20], possibly contaminating vesicles derived from mucosal scrapings.

The K^+ channel inhibitors quinidine [23], 4-aminopyridine [11] and verapamil [10] were also inhibitory in our system. The estimated I_{50} values for half-maximal inhibition of Ba^{2+}-sensitive $^{86}Rb^+$ flux obtained with the BLMS fraction were 500 μM for 4-aminopyridine, 70 μM for quinidine and 10 μM for verapamil (Fig. 4). These values are comparable to those found in other systems [10, 11, 23]. In the BLMC fraction 4-aminopyridine (3×10^{-3} M), quinidine (2×10^{-4} M), and verapamil (10^{-4} M) inhibited 27, 18, and 6% of total $^{86}Rb^+$ flux, respectively. The concentrations shown in parentheses are the maximal nondepolarizing concentrations.

Glibenclamide (10 μM), an inhibitor of ATP-

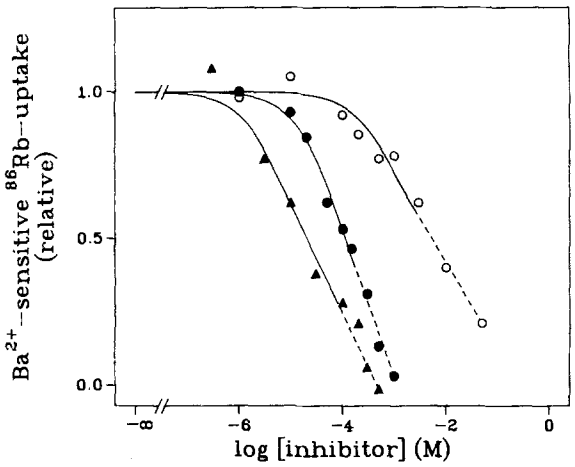

Fig. 4. Dose-response curves for the inhibition of Ba^{2+}-sensitive $^{86}Rb^-$ fluxes by quindine, 4-aminopyridine, and verapamil. Basolateral membrane vesicles of surface (BLMS) cells were assayed for Ba^{2+}-sensitive $^{86}Rb^+$ flux in the presence of increasing concentrations of quinidine (●), 4-aminopyridine (○), and verapamil (▲). Fractional uptakes as a function of inhibitor concentration (log scale) are shown. I_{50} values were estimated graphically from Eadie-Hofstee plots (% inhibition *vs.* % inhibition/inhibitor concentrations) using the nondepolarizing concentrations of the inhibitors (solid lines). The maximal nondepolarizing concentrations were determined as described in Materials and Methods. The broken lines represent inhibitory effects of the substances tested at concentrations depolarizing, at least in part, the membrane potential

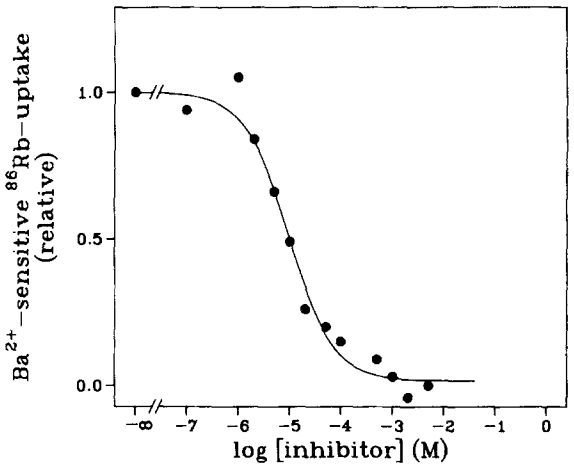

Fig. 5. Ba^{2+}-sensitive $^{86}Rb^+$ flux after reconstitution of K^+ channel activity into liposomes. K^+channel protein was solubilized from the BLMS fraction, reconstituted into liposomes and assayed for $^{86}Rb^+$ flux in the presence of increasing concentrations of Ba^{2+} (*see* Materials and Methods). Fitting the data set to Eq. (1) yields a half-maximal inhibition of 9.7 μM Ba^{2+} (residual sum of squares 0.039).

modulated K^+ channels [34] had no significant effect in either membrane fraction (*data not shown*). In both plasma membrane fractions Co^{2+} showed simple inhibition curves (*data not shown*) with half-maximal effects (Table) similar to those found in other systems [11].

Apical membranes of the surface epithelial cells in the distal colon contain amiloride-sensitive Na^+ channels [35]. It is therefore necessary to consider whether, in the absence of Na^+, $^{86}Rb^+$ could permeate through Na^+ channels [14], a process which could also be inhibited by Ba^{2+} [15]. In the conditions of our flux assays, $^{86}Rb^+$ uptake in the absence and presence of 0.1 mM amiloride (BLMS, 1080 ±95 *vs.* 1177 ± 154 cpm/10 min, n = 5; BLMC, 656 ± 152 *vs.* 679 ± 164 cpm/10 min, n = 4) were not significantly different ($P \geq 0.25$). This argues against $^{86}Rb^+$ fluxes via amiloride-sensitive Na^+channels.

The reconstitution of K^+ channels into phospholipid vesicles is essential for the identification of the channel proteins in purification procedures. Figure 5 shows the Ba^{2+}-sensitivity of $^{86}Rb^+$ flux after solubilization and reconstitution of K^+ channel activity of the BLMS fraction into phospholipid vesicles. The half-maximal inhibition at 9.7 μM Ba^{2+} in the reconstituted state is nearly identical to the value of 12 μM obtained in the native state (*see* Table). Whereas in the native state right side-out vesicles predominate [41], the liposomes are expected to contain channels predominantely with their cytoplasmic aspects facing outward [23]. This suggests that the effect of Ba^{2+} is the same whether it acts from the cytoplasm or at the extracellular membrane surface. The reconstitution technique provides the means for directly studying the modulation of channel activity by intracellular substances such as Ca^{2+}, calmodulin, and protein kinase-dependent phosphorylation and dephosphorylation.

Discussion

This study identifies Ca^{2+}-activated K^+ channels with different properties in vesicles of the surface and crypt cells derived from basolateral membranes from the rabbit distal colon epithelium. K^+channels from both membrane fractions are stimulated by Ca^{2+} in physiological concentrations (Fig. 1), but the inhibition patterns show that the properties of K^+channels found in surface cell vesicles are different from those in crypt cell vesicles. This supports the conclusion that the vesicles indeed originate from different cells in the surface and crypt area of the distal colon epithelium, respectively [41]. This

is the first significant difference with respect to the properties of ion transport systems detected in these vesicle preparations, since other ion transport systems are similar in both membrane fractions [41, 42].

The half-maximal inhibition of the K^+ channels by specific peptide toxins in the nanomolar range (Table) and the half-maximal activation at 0.5 μM free Ca^{2+} (Fig. 1) correspond to the concentrations determined in single-channel recordings in other tissues [*cf.* Ref. in 25]. This indicates that potential-driven isotope fluxes in the given vesicle preparations represent an adequate experimental system for the studies of K^+ channel properties.

The bee venom peptide, apamin, was effective as inhibitor exclusively in surface cell derived vesicles. The scorpion venom peptide, charybdotoxin, in contrast, inhibited predominately in the crypt cell derived membrane fraction and less in the surface cell derived membrane vesicles. Patch-clamp recording techniques have identified two major groups of Ca^{2+}-activated K^+ channels in mammals [30]. First, a group of large-conductance (~200 pS) K^+ channels which are blocked by charybdotoxin. Second, a small-conductance (10–14 pS) K^+ channel which is specifically blocked by apamin. The apamin- and charybdotoxin-sensitive $^{86}Rb^+$ fluxes thus reflect the presence of small- and large-conductance type Ca^{2+}-activated K^+ channels in the BLMS and the BLMC fraction, respectively.

It must be stressed that the fractions of apamin- and charybdotoxin-inhibited $^{86}Rb^+$ fluxes shown in Fig. 2 do not necessarily reflect the relative abundance of the peptide toxin-sensitive K^+ channels in the entire membrane fraction. They rather represent lower limit values, since in everted vesicles the external binding sites for the toxins [4, 27] are shielded and K^+ channels in leaky vesicles are not identified in $^{86}Rb^+$uptake studies. The orientation of the vesicles in the membrane fractions used in the present experiments approximates a sealed right side-out : sealed inside-out : leaky ratio of 2 : 1 : 1 [41].

Titrations with TEA and protons also show different properties of K^+ channels in surface and crypt cell vesicles. In the BLMS fraction, the TEA dose-response curve (Fig. 2) indicates the presence of both high- and low-sensitive $^{86}Rb^+$ flux components. By contrast, the high-sensitive component is absent in the BLMC fraction. The sensitivities to TEA (Table) are within the range previously observed in K^+ channels of different cells [*cf.* Ref. in 9, 11, 24, 25].

Acidifying the assay medium from pH 7 to 5.1 progressively inhibits $^{86}Rb^+$ fluxes with an apparent pK of 5.8 in both surface and crypt cell derived vesicles (Fig. 3). Previous titrations of various types of K^+ channels show a similar behavior in the acidic range [*cf.* Ref. in 23]. A shift of the assay pH to the low alkaline range exclusively increased the Ba^{2+}-sensitive $^{86}Rb^+$fluxes in surface cell fractions. One possible explanation is that deprotonation of relevant group(s) located at the channel protein or the surrounding lipids stimulates $^{86}Rb^+$ permeation through a certain K^+channel population. An increase in negative surface charge near the mouth of the channel could increase the local cation concentration and increase the conductance of the channels [29].

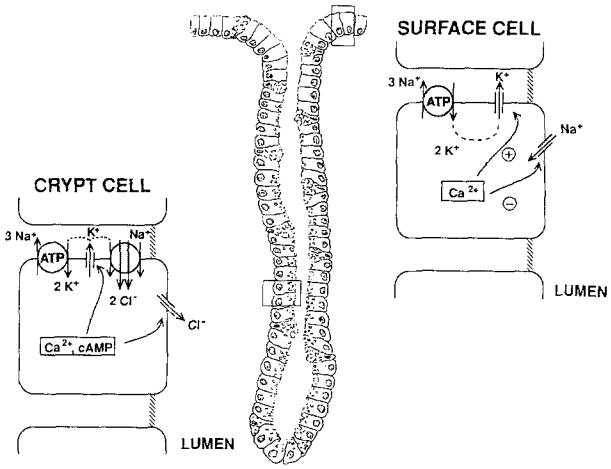

Fig. 6. Localization of Ca^{2+}-activated K^+ channels in surface and crypt cells of the rabbit distal colon epithelium with schemes for the bidirectional ion transport processes. In this model different subtypes of Ca^{2+}-activated K^+ channels are suggested, charybdotoxin-sensitive K^+ channels being present in the crypt cells, apamin-sensitive K^+ channels in the surface cells. *See* text for details

The plasma membrane fractions used in this study are highly enriched in basolateral membranes [41] and tentatively we can assign the K^+channels found to the basolateral cell domain of distal colon epithelial cells. However, we cannot exclude some background contamination by apical membranes which are proposed to have a K^+conductance in the colon epithelium [43]. It has been shown in intact tissues [17, 32] that elevation of intracellular cyclic nucleotide concentrations induces K^+ secretion which is inhibited by mucosal (apical) addition of Ba^{2+}. On the other hand, the half-maximal inhibitory effects of mucosal Ba^{2+} were in the millimolar range [17]. This is two orders of magnitude higher than the values we found in our basolateral membrane vesicle preparation (Table).

The localization of the Ca^{2+}-activated K^+channels in the surface and crypt cells of the distal colon epithelium is illustrated in Fig. 6 as basis for a discussion of their probable physiological role.

In the mammalian colon, surface epithelial cells are responsible for electrogenic Na^+ absorption

[35]. Na^+ enters the cell at the apical membrane through amiloride-blockable Na^+ channels and is extruded at the basolateral membrane via the Na,K pump. K^+ that is pumped into the cell is recycled across the basolateral membrane through conductive channels. To maintain cellular ionic and volume homeostasis during Na^+ absorption, the surface epithelial cells must continuously adjust the passive ion permeabilities of their apical and basolateral membrane as the rate of transport varies. It has been proposed [*cf.* Ref. in 7] that intracellular Ca^{2+} could play an important role in the coordination of these processes, since it activates certain types of K^+ channels [20], but inhibits amiloride-sensitive Na^+ channels [15]. Our finding that Ca^{2+}-activated K^+ channels are indeed present in basolateral membrane vesicles of surface cells would support this model.

Elevation of intracellular cAMP and Ca^{2+} stimulates electrolyte and fluid secretion in the distal colon [17, 28, 32, 40], and apically vacuolated epithelial cells in the crypts are proposed to be responsible for this process [40]. The demonstration of the Ca^{2+}-activated K^+ channel in the crypt cell basolateral membrane fraction is consistent with the general model proposed for Cl^- secreting epithelia [31]. Secretagogue-evoked Cl^- secretion results from the stimulation of the basolateral Ca^{2+}-activated K^+ channel and a putative apical anion channel [18] via intracellular messengers. Cl^- is driven into the epithelial cell against its electrochemical gradient via the basolateral Na,K,Cl-cotransport system [42] utilizing the electrochemical gradient for Na^+ generated by the Na,K pump [41]. At the apical cell domain Cl^- is released by electrodiffusion and K^+ is recycled at the basolateral membrane by the Na,K pump and/or the Na,K,Cl-cotransport system.

Crypt cells are the progenitors of the surface cells [6] and, as they move to the surface of the mucosa, their ability of electrolyte secretion is seemingly lost while absorption capability develops [40]. Our data support the idea that the expression of different subtypes of Ca^{2+}-activated K^+ channels in the basolateral cell domain of crypt and surface cells may reflect this change in their physiological function.

The important question is whether the Ca^{2+} activities required for opening of the charybdotoxin- and apamin-sensitive channels actually corresponds to Ca^{2+} activities prevailing in the crypt or surface cells of the rabbit colon.

In secretory cells, maxi K^+ channels have been found to be open at normal values of intracellular Ca^{2+} activity. In contrast, it has been concluded from work on Ca^{2+}-activated K^+-channels in the leaky epithelium of the choroid plexus [8] that intracellular Ca^{2+} activities are much lower than those required for activation. After reconstitution into planar lipid bilayer membranes consisting of phosphatidyl-ethanolamine and phosphatidyl-serine, the K^+ channels from the rabbit distal colon epithelium displayed considerable variability in their response to Ca^{2+} in the range of 1–10 μM [38]. The present results obtained with native membrane vesicles reveal a higher sensitivity to Ca^{2+} with stimulation in a range from 100 nM to 1 μM free Ca^{2+}. This is close to the estimated range of basal Ca^{2+} activity (50–100 nM) in rabbit distal colonic epithelial cells [33]. Recently, single channel recordings from excised membrane patches of rabbit distal colonocytes have shown that K^+ channel activities are increased markedly in the presence of 500 nM Ca^{2+} [26]. This corresponds well to the stimulatory concentrations of Ca^{2+} we have found in potential-driven $^{86}Rb^+$ fluxes in the given vesicle preparations. The question should therefore be raised whether the discrepancy in Ca^{2+} sensitivity can be due to the special conditions of the channel assay in the vesicle preparations.

In the present study, the membrane potential imposed during the channel assay by the KCl gradient is oriented such that the vesicle interior is negative with respect to the extravesicular solution. Membrane potential is known to reduce the open probability of Ca^{2+}-activated K^+ channels [8, 38] as the cell interior becomes more negative with respect to the extracytoplasmic solution. In the preparations used for this study, the ratio of sealed right side-out to sealed inside-out vesicles is about 2 : 1 [41]. The potential imposed by the KCl gradient during the assay of K^+ channel activity may thus increase the open probability for channels in inside-out vesicles, but in the majority of the membrane vesicles, the potential would reduce the open probability at a given Ca^{2+} activity. In the intact cell, the response to Ca^{2+} is also modified by regulatory signals. Thus, cAMP activate K^+ channels in patches of basolateral membranes from crypt cells of the rabbit distal colon [26]. After reconstitution, an activation of Ca^{2+}-dependent K^+ channels from the outer renal medulla was observed after cAMP-dependent protein kinase catalyzed phosphorylation from ATP [23]. These reactions should be taken into account in evaluating the possible physiological role of the K^+channels in the distal colon epithelium. To resolve this complex situation, a detailed quantitative analysis using electrophysiological techniques will be required to determine the Ca^{2+} activation of each of the K^+channel types in the vesicle preparations from both surface and crypt cells in the colon epithelium.

4-Aminopyridine [9, 19] and verapamil [21, 36]

are not or at most weakly inhibitory for Ca^{2+}-activated K^+channels, although they are highly effective with other types of K^+ channels [11, 10]. Both substances were inhibitory in our experimental system. This indicates that other types of K^+ channels might coexist in the given membrane fractions, in addition to the Ca^{2+}-activated K^+ channels. Verapamil inhibits K^+ secretion in the colon epithelium, and this has been used as an argument that Ca^{2+} influx via putative Ca^{2+} channels seems to play a role in K^+ secretion [32]. This would stimulate the question whether Ca^{2+} channels could account for part of the $^{86}Rb^+$ fluxes, since Ca^{2+} channels allow monovalent cations to permeate when Ca^{2+} ions are absent [13]; we do not favor the idea that Ca^{2+} channels might mediate part of the $^{86}Rb^+$ fluxes in the given membrane fractions. First, the monovalent cation current of Ca^{2+} is inhibitory to $^{86}Rb^+$ fluxes up to a concentration of 10 μM (Fig. 1). Second, prior to the assay of $^{86}Rb^+$ fluxes, the vesicles were routinely preincubated in a medium containing millimolar concentrations of Mg^{2+} along with other constituents to inhibit Na,K-ATPase [23]. Even when Ca^{2+} channels were present we would probably have missed them since Mg^{2+} blocks (I_{50} = 30 μM) the monovalent cation current of Ca^{2+} channels [13].

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