Quantification and distribution of Ca\textsuperscript{2+}-activated maxi K\textsuperscript{+} channels in rabbit distal colon

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Quantification and distribution of Ca\textsuperscript{2+}-activated maxi K\textsuperscript{+} channels in rabbit distal colon. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G22-G30, 1999.—The Ca\textsuperscript{2+}-activated maxi K\textsuperscript{+} channel is an abundant channel type in the distal colon epithelium, but nothing is known regarding the actual number and precise localization of these channels. The aim of this study has therefore been to quantify the maxi K\textsuperscript{+} channels in colon epithelium by binding of iberiotoxin (IbTX), a selective peptidyl ligand for maxi K\textsuperscript{+} channels. In isotope flux measurements 75% of the total K\textsuperscript{+} channel activity in plasma membranes from distal colon epithelium is inhibited by IbTX ($K_{D}$ = 4.5 pM), indicating that the maxi K\textsuperscript{+} channel is the predominant channel type in this epithelium. Consistent with the functional studies, the radiolabeled double mutant $^{125}$I-IbTX-D19Y/Y36F binds to the colon epithelium membranes with an equilibrium dissociation constant of ~10 pM. The maximum receptor concentration values (in fmol/mg protein) for $^{125}$I-IbTX-D19Y/Y36F binding to colon epithelium are 78 for surface membranes and 8 for crypt membranes, suggesting that the maxi K\textsuperscript{+} channels are predominantly expressed in the Na\textsuperscript{+}-absorbing surface cells, as compared with the Cl\textsuperscript{-}-secreting crypt cells. However, aldosterone stimulation of this tissue induced by a low-Na\textsuperscript{+} diet does not change the total number of maxi K\textsuperscript{+} channels.

THE WATER AND SALT HOMEOSTASIS of the organism is partly maintained by regulated absorption and secretion of NaCl across specialized epithelial cells in the distal colon (for reviews, see Refs. 6 and 16). In this epithelium, the surface cells are mainly responsible for the aldosterone-stimulated Na\textsuperscript{+} reabsorption through luminal amiloride-sensitive Na\textsuperscript{+} channels, while Cl\textsuperscript{-} is secreted through Cl\textsuperscript{-} channels in the luminal membrane of the crypt cells (18, 35). In both cell types, the transepithelial transport is mediated by the collaboration of several membrane transport systems, including the Na\textsuperscript{+}-K\textsuperscript{+} pump, the Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport system, and K\textsuperscript{+} channels. The Na\textsuperscript{+}-K\textsuperscript{+} pump in the basolateral membrane delivers the primary driving force for the transport by creating an electrochemical gradient for Na\textsuperscript{+}, whereas basolateral K\textsuperscript{+} channels allow K\textsuperscript{+} to recycle across this membrane to maintain the intracellular K\textsuperscript{+} homeostasis (7). Therefore, the transepithelial ion transport requires strict regulation of the basolateral K\textsuperscript{+} channels, e.g., during hormonal stimulation of the tissue.

In vesicle preparations of the basolateral membrane of surface cells, the predominant channel type is a Ca\textsuperscript{2+}-activated maxi K\textsuperscript{+} channel with a single-channel conductance of ~275 pS (21, 39). Single-channel studies have shown that the epithelial maxi K\textsuperscript{+} channels are tightly regulated by, for instance, membrane potential, intracellular Ca\textsuperscript{2+}, phosphorylation/dephosphorylation, and nucleotides (23, 24, 41). It is therefore obvious to suggest that this channel may play an important role in the regulation of the transepithelial transport in the surface cells. However, in patch-clamp studies, this channel type has also been identified in colonocytes originating from crypt cells (2, 28, 30, 36). Because of the lack of suitable ligands, it has not so far been possible to determine the relative abundance and the precise localization of the maxi K\textsuperscript{+} channels in the distal colon epithelium. As a consequence, it is also not known if the expression of these channels is modulated in response to hormonal stimulation of the tissue.

Ca\textsuperscript{2+}-activated maxi K\textsuperscript{+} channels from a number of tissues have been shown to be sensitive to extracellular application of peptidyl and nonpeptidyl agents, e.g., Ba\textsuperscript{2+} (31), tetraethylammonium (TEA) (40), charybdoxin (ChTX) (29), and iberiotoxin (IbTX) (10), which prevent K\textsuperscript{+} flux by a physical occlusion of the pore. IbTX is a toxin consisting of 37 amino acids isolated from the venom of the scorpion, Buthus tamulus, and this toxin has, in contrast to other peptides, been shown to be a selective, high-affinity blocker of Ca\textsuperscript{2+}-activated maxi K\textsuperscript{+} channels from excitatory tissue (10). Measurements of IbTX binding to the K\textsuperscript{+} channels were originally hampered by the fact that the native IbTX loses its biological activity upon iodination (26). Recently, however, a double mutant of this toxin, IbTX-D19Y/Y36F, has been constructed and iodinated with full preservation of the affinity and selectivity for maxi K\textsuperscript{+} channels (26).

In the present study we show that, as in excitatory tissue, IbTX comprises a high-affinity blocker of maxi K\textsuperscript{+} channels in epithelia. For the first time the radiolabeled ligand $^{125}$I-IbTX-D19Y/Y36F now gives us the opportunity not only to determine the abundance of maxi K\textsuperscript{+} channels in epithelia but also to obtain some information about their localization in distal colon. In addition, the $^{125}$I-IbTX-D19Y/Y36F binding studies allow us to examine whether the expression of maxi K\textsuperscript{+} channels in distal colon epithelium is modulated by aldosterone stimulation.

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**EXPERIMENTAL PROCEDURES**

**Materials**

Tris, MOPS, EGTA, BSA, sucrose, 3-[3-chloroamidopropyl]-dimethylammonio]-1-propanesulfonate (CHAPS), polyethyleneimine, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma; 1,4-dithiothreitol (DTT) was from Boehringer Mannheim; KCl, NaCl, BaCl2, and MgCl2 were from Merck; Digitonin was from Serva; polysulfone tubes were from InterMed; GC 50 filters were from Toyo Advantec; ouabain and Dowex beads (50 W × 8) were from Fluka. The Dowex beads were converted to the Tris form by 16–24 h of incubation with Tris base (suspension pH > 10) and subsequent repetitive washing with distilled water until the suspension pH dropped to 7.0–7.4. Strontium chloride (SrCl2) (0.5–12 mCi/mg mg Sr+) was from Amersham. Purification and ionization of 125I-labeled Y 36F (sp act 2.175 Ci/mmol) were done as previously described (26).

**Tissue Preparation**

New Zealand White rabbits (female, ~2.5 kg) maintained on normal rabbit chow and tap water or on a low-Na+ diet consisting of barley (0.003% Na+ and 0.33% K+) plus distilled water were killed by cervical dislocation and bled. The distal colon was immediately dissected, cut into pieces of ~3 cm, flushed with 0.9% NaCl to remove fecal contents, and placed on ice. The segments were then opened along their length and placed with the luminal side upward on a 150-mm cooled petri dish. After the colon was cleaned thoroughly with gauze to remove mucus, the colon mucosa was harvested by gentle scraping with two glass slides (44). The colon mucosa was then placed in liquid nitrogen and stored at -80°C.

**Vesicle Preparation**

Tissue scrapings (2 g) were homogenized either with 30 ml of 250 mM sucrose, 1 mM EGTA, and 10 mM MOPS-Tris, pH 7.0 (for binding experiments), or with 30 ml of 250 mM sucrose, 50 mM KCl, 1 mM EGTA, and 10 mM MOPS-Tris, pH 7.2 (for flux experiments) with 10 strokes at 1,000 rpm in a glass/Teflon homogenizer (Braun-Melsungen) at 0°C. In both cases, 1 mM DTT and 0.2 mM PMSF were added just before homogenization. The homogenate was subjected to low-speed centrifugation. The supernatant, containing soluble proteins and crude membrane fractions, was decanted and discarded, and mucus from the inner side of the centrifuge glasses was removed before the pellet was resuspended in the cold buffer consisting of 150 mM NaCl, 0.1% BSA, and 20 mM Tris·HCl, pH 7.4, in 10 × 75 mm polystyrene tubes. Nonspecific binding was defined in the presence of 10 nM IBTX, and incubation was carried out at room temperature for 15–24 h (demasking experiments) or 60–72 h (saturation experiments). All serial toxin dilutions were performed in 0.5 ml medium consisting of 10 mM NaCl, 0.1% BSA, and 20 mM Tris·HCl, pH 7.4, and were always added directly to the incubation medium to avoid adsorption phenomena. Detergent was allowed to incubate with the membranes for 20 min at room temperature before the ligand was added. The protein concentration of the membrane vesicles was 20–100 µg/ml for demasking experiments and 13–50 µg/ml for saturation experiments. At the end of incubation the samples were rapidly filtered through Toyo Advantec GC 50 glass fiber filters [presoaked for at least 60 min in 0.3% (wt/vol) polyethyleneimine] on a Millipore 1002530 filter apparatus, followed by two washes with ice-cold buffer consisting of 150 mM NaCl and 20 mM Tris·HCl, pH 7.4 (3 ml/wash). In each experiment triplicate assays were routinely performed and the data were averaged.

**Flux Assay**

The total K+ channel activity in plasma membrane vesicles was measured as the Ba2+-sensitive potential-driven 86Rb+ uptake at 25°C that could be blocked by 4.4 mM BaCl2, as described earlier (22). To minimize a possible background flux due to the Na+-K+ pump, the vesicles were preincubated with 0.5 mM ouabain, 0.1 mM vanadate, and 0.5 mM MgCl2 for 30 min. Furosemide was added to minimize a possible nonspecific flux through the Na+-2Cl−-K+ cotransport system present in the distal colon epithelium. In experiments in which the effect of IBTX was examined, the vesicles were then preincubated for 30 min with different concentrations (10−15–10−7 M) of toxin before the Ba2+-sensitive 86Rb+ uptake (40 min) was measured.

**Protein Determination**

The concentration of membrane protein was determined according to Peterson (33), using BSA as a standard.

**Analysis of Data**

Radioligand binding studies. The results from saturation binding experiments were subject to a Michaelis-Menten analysis in which the equilibrium dissociation constant (Kd) and the maximal receptor concentration (Bmax) were determined using the one-site binding equation Y = Bmax·X/(Kd + X), where Y is receptor concentration and X is radioligand concentration. The correlation coefficient for these plots
was $>0.97$. The data from saturation experiments were transformed into Scatchard plots, and the $B_{\text{max}}$ and $K_d$ values determined from this linear regression were nearly identical to the values determined from the one-site binding equation.

Flux assay. The results from the flux assay experiment were subject to the one-site competition equation $Y = (\text{maximal binding} - \text{minimal binding})/(1 + 10^{X-\log K_d})$, where $Y$ is binding and $X$ is concentration of radioligand (log). The correlation coefficient from this plot was 0.99.

Statistics

All data points are given as means ($n \geq 3$). The exact numbers are indicated in relevant figures. Standard deviations from the mean are given as error bars.

RESULTS

Inhibition of Epithelial Ca$^{2+}$-Activated Maxi K$^+$ Channels by IbTX

For an initial investigation of the ability of IbTX to interact with epithelial Ca$^{2+}$-activated maxi K$^+$ channels at a single-channel level, crude plasma membrane vesicles from the distal colon epithelium were fused with planar lipid bilayers. Consistent with earlier studies (for review, see Ref. 21), the predominant channel was a Ca$^{2+}$-activated maxi K$^+$ channel with a single-channel conductance of $\approx 275$ pS (Fig. 1). Addition of IbTX to the outside of the maxi K$^+$ channel blocked the channel after a delay of $\approx 10$ min, whereas addition of IbTX to the intracellular face had no effect (data not shown). The delay in the observed effect of IbTX is due to the relatively slow association constant (data not shown). The delay in the observed effect of IbTX is due to the relatively slow association constant (data not shown). The delay in the observed effect of IbTX is due to the relatively slow association constant (data not shown). The delay in the observed effect of IbTX is due to the relatively slow association constant (data not shown). The delay in the observed effect of IbTX is due to the relatively slow association constant (data not shown).

A

Fig. 1. Single-channel recordings of Ca$^{2+}$-activated K$^+$ channels in lipid bilayers. A: crude membrane vesicles from rabbit distal colon were fused with planar lipid bilayers at a membrane potential of $-40$ mV and a free Ca$^{2+}$ concentration of $200 \mu M$ to incorporate the epithelial Ca$^{2+}$-activated maxi K$^+$ channel with a single-channel conductance of $\approx 275$ pS. B: Iberiotoxin (IbTX; 75 nM) has been added to the extracellular face of the channel.

These measurements indicate that IbTX is in fact an effective blocker of epithelial maxi K$^+$ channels from the outside.

Although the high incorporation rate of the maxi K$^+$ channels from the colon vesicle preparation gives some indication that these channels are rather abundant in this epithelium, the single-channel measurements must be considered strictly qualitative. To obtain some information concerning the abundance of maxi K$^+$ channels in comparison with other types of K$^+$ channels in the colon epithelium, we employed a sensitive flux assay, which has earlier proven very useful for measurement of ion channels in epithelial plasma membrane vesicle preparations (13, 22, 42). To overcome the problems of measuring fast channel-mediated isotope fluxes in a heterogeneous population of membrane vesicles with a relatively low density of channels, this assay measures the potential-driven $^{86}$Rb$^+$ uptake into the plasma membrane vesicles in the presence or absence of K$^+$ channel inhibitors.

Figure 2A shows an experiment in which the $^{86}$Rb$^+$ uptake into the crude colon membrane vesicles has been measured under three conditions: in control membranes (no addition) and after addition of either 10 nM IbTX or 4.4 mM BaCl$_2$. The free concentration of Ca$^{2+}$ is $200 \mu M$, which ensures that the maxi K$^+$ channels are open even at negative membrane potentials (Fig. 1). Under these conditions Ba$^{2+}$ blocked $\approx 40\%$ of the $^{86}$Rb$^+$ uptake, and, because Ba$^{2+}$ in millimolar concentrations is an inhibitor of practically all types of K$^+$ channels, the Ba$^{2+}$-sensitive fraction of the $^{86}$Rb$^+$ uptake into the vesicles can be considered a measure for the total K$^+$ channel activity in the membrane vesicle preparation. IbTX blocked $\approx 30\%$ of the total $^{86}$Rb$^+$ uptake, corresponding to $\approx 75\%$ of the Ba$^{2+}$-sensitive $^{86}$Rb$^+$ uptake.

To estimate the affinity for the inhibitory effect of IbTX, the Ba$^{2+}$-sensitive $^{86}$Rb$^+$ uptake into the colon vesicles was measured in the presence of increasing concentrations of IbTX. Under these conditions an apparent $K_{0.5}$ of 4.5 nM was determined (Fig. 2B). The fact that IbTX blocks the epithelial maxi K$^+$ channels with a high affinity makes this peptide a suitable ligand for binding studies in this tissue.

Binding of $^{125}$I-IbTX-D19Y/Y36F to Epithelial Membranes

For binding studies the radiolabeled IbTX analog, $^{125}$I-IbTX-D19Y/Y36F, was employed (26). Figure 3 shows an initial experiment in which binding of $^{125}$I-IbTX-D19Y/Y36F to different amounts of crude colon vesicles was measured. The specific binding was dependent on the amount of vesicles (measured as µg membrane protein), and it constituted $\approx 75$–$80\%$ of the total binding.

Considering the fact that IbTX is only able to interact with the extracellular part of the Ca$^{2+}$-activated K$^+$ channel, and that it must be expected that a certain part of the vesicles in the plasma membrane preparation are oriented inside out, some $^{125}$I-IbTX-D19Y/Y36F binding sites may not readily be accessible in binding experiments. To ensure a reliable quantification of the
Ca²⁺-activated maxi K⁺ channels by [¹²⁵]IbTX-D19Y/Y36F binding, these binding sites must be demasked by opening of the plasma membrane vesicles through incubation with a suitable detergent.

Three different detergents, digitonin, CHAPS, and sodium deoxycholate, were examined. Initial experiments showed that CHAPS and sodium deoxycholate, even in low concentrations, interfered with the binding of [¹²⁵]IbTX-D19Y/Y36F (data not shown), and for this reason these detergents were not further investigated in the experiments described below. Figure 4 shows the results of experiments in which the binding of [¹²⁵]IbTX-D19Y/Y36F to the colon vesicles was measured in the presence of increasing concentrations of digitonin. It is evident that maximal binding was found in the presence of a detergent-to-protein ratio of 0.5–0.75 mg digitonin/mg protein. Demasking of the vesicles by detergent increases the maximal binding by 23% compared with control, indicating that ~20% of the vesicles are oriented inside out, whereas ~80% of the vesicles are either right side out or nonsealed. This finding corresponds nicely with previous data, in which this membrane preparation was shown to exhibit a vesicle orientation with an approximately right side out-to-inside out-to-leaky ratio of 2:1:1 (44). The fact that binding of [¹²⁵]IbTX-D19Y/Y36F to totally demasked membrane vesicles can be measured strongly supports the suitability of this ligand in studies on quantification of Ca²⁺-activated maxi K⁺ channels.

At detergent concentrations exceeding 5 mg digitonin/mg membrane protein, the specific [¹²⁵]IbTX-D19Y/Y36F binding decreased significantly (Fig. 4). This is presumably due to the fact that the presence of high detergent concentrations compromises filter retention of solubilized channel protein.

All further experiments were performed in the presence of 0.625 mg digitonin/mg membrane protein to ensure full access of [¹²⁵]IbTX-D19Y/Y36F to its binding site in the external channel vestibule.

Figure 5 shows association and dissociation curves for [¹²⁵]IbTX-D19Y/Y36F binding to the crude plasma membrane fraction from the colon epithelium. Holding the receptor concentration below and the ligand concentration above equilibrium, the [¹²⁵]IbTX-D19Y/Y36F binding isotherm was fitted to a 1-site competition equation, and the $K_{0.5}$ for the IbTX inhibition was determined to be 4.5 pM. All data points are given as means ± SE ($n = 4$).
tration around $K_d$, the kinetic rates were relatively slow at room temperature; the association was half completed after 2 h and equilibrium binding conditions were achieved after $\sim 10$ h. For the dissociation the apparent $K_{0.5}$ was $\sim 8$ h. At 0°C the dissociation was even slower, and we did not observe significant ligand dissociation within 30 h (data not shown). These data correspond nicely to the parameters determined for rat smooth muscle, in which the association and dissociation at room temperature occurred with half-maximal times of 1.5 and 8 h, respectively (26).

Quantification of Ca$^{2+}$-Activated Maxi K$^+$ Channels in Rabbit Distal Colon after Aldosterone Stimulation

Studies on whole epithelia have shown that the K$^+$ conductance in the basolateral membrane of the epithelial cells in colon is increased when the transepithelial transport is upregulated, e.g., during hormonal stimulation of the tissue with aldosterone (26). So far it is not established whether such a stimulation involves an increased expression of maxi K$^+$ channels. To stimulate the distal colon epithelium in vivo, rabbits were kept on a low-Na$^+$ diet consisting of pearl barley and distilled...
water for up to 7 days. This procedure is known to markedly enhance the endogenous secretion of aldosterone, resulting in an increase in plasma aldosterone levels of more than threefold compared with control levels (43).

To determine the number of maxi K⁺ channels in the epithelium after hormonal stimulation, rabbits were killed after 2 or 7 days on the low-Na⁺ diet, and the number of ¹²⁵I-1bTX-D19Y/Y36F binding sites was measured in crude vesicle preparations of the distal colon. The results are shown in Fig. 8, and as can be seen there is no significant change in the number of binding sites in membrane vesicles prepared from the colon of stimulated animals compared with those prepared from control animals. These measurements indicate that aldosterone stimulation of the distal colon epithelium does not alter the expression level of Ca²⁺-activated maxi K⁺ channels.

**DISCUSSION**

**Inhibition of Epithelial Maxi K⁺ Channels by 1bTX**

In many cases toxins have proven to be valuable tools to study ion channels. In the characterization of Ca²⁺-activated maxi K⁺ channels, two peptidyl scorpion toxins of 37 amino acids, ChTX and IbTX, have been particularly interesting. The toxins share a homology of 68%, and their interaction with the outer vestibule of the maxi K⁺ channels from excitatory tissues, such as muscle and the central nervous system, has been studied extensively (4, 12, 14, 37). Recent studies have shown that ChTX is not specific for maxi K⁺ channels.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Bmax, pmol/mg protein</th>
<th>Kd, pM</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface membrane</td>
<td>0.078 ± 0.004</td>
<td>9.68 ± 1.38</td>
<td>This study</td>
</tr>
<tr>
<td>Crude membrane</td>
<td>0.041 ± 0.003</td>
<td>22.56 ± 3.34</td>
<td>This study</td>
</tr>
<tr>
<td>Crypt membrane</td>
<td>0.008 ± 0.001</td>
<td>8.52 ± 3.35</td>
<td>This study</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.141</td>
<td>23.3</td>
<td>Ref. 25</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.029</td>
<td>19.1</td>
<td>Ref. 25</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>0.420</td>
<td>5.2</td>
<td>Ref. 25</td>
</tr>
</tbody>
</table>

Values for membrane tissues are means ± SD. Distribution of Ca²⁺-activated maxi K⁺ channels was determined by ¹²⁵I-1bTX-D19Y/Y36F binding (this study) and by ³H-IbTX-D19C binding (Ref. 25). Bmax, maximum receptor concentration; Kd, equilibrium dissociation constant.
but in addition blocks the intermediate conductance Ca\(^{2+}\)-activated K\(^+\) channels (20) and voltage-gated K\(^+\) channels (15). In contrast, IbTX is not known to block other types of K\(^+\) channels and therefore seems to be a suitable ligand to characterize maxi K\(^+\) channels, at least in excitatory tissues (10, 11).

The molecular properties of maxi K\(^+\) channels are known to vary from tissue to tissue; e.g., it has been found that the Ca\(^{2+}\) sensitivity for epithelial maxi K\(^+\) channels is considerably higher than the Ca\(^{2+}\) sensitivity for maxi K\(^+\) channels from excitatory tissues and that some maxi K\(^+\) channels are upregulated by phosphorylation, whereas others are downregulated (34; for review, see Ref. 21). Because of this tissue-to-tissue variation, it was not a priori clear whether IbTX would act as a high-affinity blocker for the epithelial maxi K\(^+\) channels. The experiments in Figs. 1 and 2 show that IbTX does indeed block maxi K\(^+\) channels from rabbit distal colon with high affinity (K\(_{0.5}\) = 4.5 nM), which is comparable to that found for excitatory tissue (4, 14). These data therefore indicate that, although the regulatory properties of maxi K\(^+\) channels may vary, the pore region is well conserved.

Figure 3 shows that 75-80% of the Ba\(^{2+}\)-sensitive \(^{86}\)Rb\(^+\) uptake into crude plasma membrane vesicles prepared from rabbit distal colon epithelium is blocked by IbTX. If it is assumed that the Ba\(^{2+}\)-sensitive \(^{86}\)Rb\(^+\) uptake represents the activity of all types of K\(^+\) channels present in these vesicles and that the IbTX-inhibited uptake represents the activity of maxi K\(^+\) channels, these data indicate that maxi K\(^+\) channels constitute a major fraction of the total population of K\(^+\) channels in the colon epithelium. This is in good accordance with our earlier flux studies, showing that the major part of the K\(^+\) channel activity in basolateral plasma membrane vesicles from colon epithelium is sensitive to changes in intracellular Ca\(^{2+}\) (42). However, an exact quantification of channel densities can never be based on isotope flux measurements, because this potential-driven assay is rather indirect and IbTX will only block channels in right side out orientation.

Binding of IbTX to Epithelial Maxi K\(^+\) Channels

The apparent specificity and high affinity of IbTX inhibition of epithelial maxi K\(^+\) channels suggest that this peptide could be an ideal ligand for binding studies. Native IbTX contains only a single tyrosine residue in position 36, but iodination of this amino acid leaves the peptide biologically inactive. This is probably due to the fact that tyrosine-36 composes part of the toxin’s interaction surface with the maxi K\(^+\) channel. Recently, it has been shown that substitution of tyrosine in position 36 by phenylalanine and introduction of a new tyrosine in position 19 rendered the double-mutated toxin with full biological activity (26). In addition, the mutated toxin, IbTX-D19Y/Y36F, can be iodinated at tyrosine-19 without loss of the biological activity (26), and this ligand is therefore used in the binding experiments on membranes from rabbit distal colon epithelium.

The binding experiments were carried out with three different membrane fractions derived from the rabbit distal colon, which were prepared according to the procedure developed by Wiener et al. (44). The crude membrane fraction contains membrane vesicles from the whole colon epithelium, i.e., from surface cells as well as crypt cells, whereas the surface membranes and the crypt membranes are highly purified in basolateral plasma membranes from the surface and crypt cells, respectively. In all cases the \(^{125}\)I-IbTX-D19Y/Y36F binding is a saturable function of the ligand concentration (Fig. 5). The respective Scatchard transformations result in linear plots consistent with the idea that the toxin interacts with one binding site on the maxi K\(^+\) channels, i.e., the external channel pore. The dissociation constant for the binding of \(^{125}\)I-IbTX-D19Y/Y36F to the membrane vesicles ranges from 6 to 15 pM, which corresponds nicely with the K\(_{0.5}\) found for the inhibition of the K\(^+\) channels measured in the flux assay (compare Fig. 3, Fig. 6, and Table 1), and this supports the notion that \(^{125}\)I-IbTX-D19Y/Y36F interacts with the channel in a manner similar to the native toxin. The equilibrium parameters as well as the kinetic constants measured for \(^{125}\)I-IbTX-D19Y/Y36F binding to epithelial membranes are comparable to those found for rat smooth muscle and brain membranes (see RESULTS and Table 1). Again, this indicates that the pore region of the maxi K\(^+\) channel is well conserved between different species and tissues.

If a 1:1 stoichiometry for \(^{125}\)I-IbTX-D19Y/Y36F binding to the maxi K\(^+\) channel is assumed, the density of the channels in the membrane vesicle preparations can be estimated directly from the B\(_{\text{max}}\) values (Table 1). In the crude membrane fraction the B\(_{\text{max}}\) is 41 fmol/mg protein. For comparison, [\(^{3}\)H]ouabain labels 12 pmol/mg protein of sites (43), suggesting that the Na\(^+\)-K\(^+\) pump is 150 times more abundant in these membranes than the maxi K\(^+\) channels. In the highly purified plasma membrane fractions, which are expected to be essentially free of contamination by intracellular membranes, the B\(_{\text{max}}\) values (in fmol/mg protein) are 78 (surface membranes) and 8 (crypt membranes), indicating that the maxi K\(^+\) channels are 10 times more abundant in the membranes from surface cells than in the crypt cell membranes.

It should be kept in mind that these data do not necessarily directly reflect the expression level in the surface and crypt cells in the intact epithelium, since the purity of the respective membrane fractions may differ. In fact, Wiener et al. (44) reported that the specific activity of the Na\(^+\)-K\(^+\)-ATPase, a marker enzyme for basolateral membranes, is higher in the surface membrane fraction than in the crypt membrane fraction. This could indicate that the surface membranes are purified to a higher degree than the crypt membranes. However, such a conclusion assumes that the densities of Na\(^+\)-K\(^+\)-ATPase in the basolateral membranes of surface and crypt cells are similar, which may not necessarily be the case. In any case, our results show that the density of maxi K\(^+\) channels in purified surface membranes is higher than in crude colon.

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membranes, whereas the density in purified crypt membranes is lower than in crude colon membranes. This finding strongly argues for a predominant expression of maxi K⁺ channels in the surface cells. In addition, preliminary immunohistochemical studies in our laboratory with the use of specific antibodies localize the maxi K⁺ channels mainly to the surface cells.

Because ion channels are generally thought to be much more abundant in excitatory tissue than in epithelia, it could be expected that the number of maxi K⁺ channels would be considerably lower in the epithelial membranes than in those from excitatory tissues. However, if the Bmax values in Table 1 are compared, it is seen that, in the colon surface membranes, the abundance of the maxi K⁺ channels is comparable to that detected in brain membranes. Although there are no direct measurements for the abundance of other channel types in epithelia, this gives some indication that the maxi K⁺ channel is indeed an abundant ion channel type in the colon surface cells, and that this channel is expected to play an important role in the function of these cells.

Physiological Role of Maxi K⁺ Channels in the Distal Colon Epithelium

In the distal colon epithelium several types of K⁺ channels have been identified (for reviews, see Refs. 7, 17, 21). According to the accepted model, surface cells are mainly responsible for aldosterone-stimulated Na⁺ reabsorption, whereas Cl⁻ secretion occurs in the crypts (35). Although amiloride-sensitive Na⁺ channels (5) and aldosterone receptors (27) are mainly expressed in the surface cells and Cl⁻ channels are mainly expressed in the crypt cells (9), recent data indicate that the boundaries between surface and crypt cells as far as function is concerned are not as distinct as formerly believed. There is now good evidence that, under certain conditions, at least midcrypt cells may change their function from Cl⁻ secretion to Na⁺ absorption and vice versa (for review, see Ref. 16).

However, the physiological function of epithelial cells in distal colon seems to be reflected in the expression of different types of K⁺ channels. In crypt cells, patch-clamp studies on the basolateral membrane have shown the expression of at least two types of K⁺ channels, a low-conductance Ca²⁺-activated K⁺ channel with a single-channel conductance of 15 pS and a Ca²⁺-insensitive, cAMP-activated K⁺ channel of ~3 pS (1, 2, 32). In addition, these cells may express K⁺ channels of the KvLQT and minK type (17). Ca²⁺-activated maxi K⁺ channels have been identified in isolated colonic crypt cells (28, 30, 36), but are only rarely seen in the basolateral membrane of crypt cells in the intact epithelium (2). In the basolateral membrane of the surface cells, our earlier data (21, 24, 42) and the present study strongly suggest that the Ca²⁺-activated maxi K⁺ channels here are expressed at high levels. In addition, patch-clamp studies have shown that maxi K⁺ channels may not only be expressed in the basolateral membrane of the surface cells but also in the luminal membrane (3).

It is now obvious to suggest that the Ca²⁺-activated maxi K⁺ channels play an important role in the function of the surface cells and that the increase in the basolateral K⁺ conductance seen in whole epithelia after aldosterone stimulation (6, 7) could be mediated through activation of Ca²⁺-activated K⁺ channels. In principle, there are three possible mechanisms for this activation: 1) an activation of channels already present in the plasma membrane, 2) a recruitment of channels situated in intracellular vesicles, or 3) an increase in the total number of channels, i.e., synthesis of new channels. To address these unresolved questions, we measured the maxi K⁺ channel density in animals after increasing the endogenous aldosterone level through dietary Na⁺ deprivation (Fig. 8). These experiments clearly show that the maxi K⁺ channel density remains unchanged after hormonal stimulation. It must therefore be expected that the increase in the K⁺ channel activity in the plasma membrane by aldosterone stimulation is due either to activation of existing channels or to enhanced incorporation of K⁺ channels into the plasma membrane from intracellular pools. Because the experiments in Fig. 8 are done on crude membranes, which contain both intracellular membranes and plasma membrane, in the present study it is not possible to discriminate between these two possibilities.

There are several possibilities for how a short-term regulation of the epithelial maxi K⁺ channels in response to aldosterone stimulation could occur: for Na⁺-absorbing epithelium in frog skin, it is known that aldosterone stimulation leads to an increase in pH (19), and, in studies on rat colon, it is shown that aldosterone stimulation mediates an increase in intracellular Ca²⁺ (8). In addition, a recent study has shown that steroid hormones may modulate the maxi K⁺ channel activity through phosphorylation and dephosphorylation (38). Because we have earlier shown that the epithelial maxi K⁺ channels are activated by alkalization, by Ca²⁺ in the intracellular range of concentration, and by phosphorylation (for review, see Ref. 21), it is quite possible that these parameters may play a crucial role in activation of basolateral K⁺ channels during hormonal stimulation of the colon epithelium.

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