Colonic ion transport is controlled by the intracellular concentration of second messengers such as Ca\(^{2+}\), cAMP and cGMP (for review see Binder & Sandle, 1994). Ca\(^{2+}\)-dependent secretagogues such as acetylcholine or its stable analogue, carbachol, induce a strong Cl\(^{-}\) secretion due to the opening of Ca\(^{2+}\)-sensitive basolateral K\(^{+}\) channels (Bleich et al. 1996). The resulting hyperpolarisation favours the exit of Cl\(^{-}\) ions due to an increase in the driving force (Böhme et al. 1991). The agonist-induced increase in the intracellular Ca\(^{2+}\) concentration usually follows a biphasic time course: an initial peak, caused by the release of Ca\(^{2+}\) from intracellular inositol-1,4,5-trisphosphate (IP\(_3\))-sensitive stores, is followed by an influx of Ca\(^{2+}\) from the extracellular medium (Lindqvist et al. 1998). The Ca\(^{2+}\) influx during the second phase of receptor-mediated Ca\(^{2+}\)-dependent secretion is in general thought to be caused by the opening of store-operated ion channels in the cell membrane, which are activated by as yet incompletely understood mechanism(s) after depletion of the intracellular Ca\(^{2+}\) stores (for review see Parekh & Penner, 1997).

Recently, we studied the properties of the store-operated cation channels in rat colonic epithelium using the whole-cell patch-clamp method. The Ca\(^{2+}\) store was depleted during whole-cell recordings by replacing the normal cytoplasm with a pipette solution containing a high concentration of the Ca\(^{2+}\) chelator, EGTA. Under these conditions, a non-selective, La\(^{3+}\)-sensitive cation conductance which was permeable to Na\(^{+}\) and Ca\(^{2+}\), but in contrast to Na\(^{+}\), Ca\(^{2+}\) also exerts a (feedback) inhibition on its own influx. Other divalent cations shared this inhibitory action with the sequence: Ca\(^{2+}\) ≥ Mg\(^{2+}\) ≥ Ba\(^{2+}\) ≥ Sr\(^{2+}\). Fura-2 experiments revealed that replacement of extracellular Na\(^{+}\) by NMDG\(^{+}\) induced an increase in the intracellular Ca\(^{2+}\) concentration, which was suppressed by the Na\(^{+}\)–Ca\(^{2+}\) exchange inhibitor, dichlorobenzamil, indicating the presence of a Na\(^{+}\)–Ca\(^{2+}\) exchanger within the colonic crypt cells. In Ussing chamber experiments dichlorobenzamil induced an increase in short-circuit current (\(I_{sc}\)) in the majority of tissues tested indicating that this exchanger acts as a Ca\(^{2+}\)-extruding transporter under physiological conditions. When Ca\(^{2+}\)-dependent anion secretion was stimulated by the acetylcholine analogue carbachol, dichlorobenzamil no longer evoked an increase in \(I_{sc}\), indicating that after stimulation of the store-operated cation conductance the Na\(^{+}\)–Ca\(^{2+}\) exchanger is turned off. Therefore, it is concluded that the influx of Na\(^{+}\) across the non-selective store-operated cation conductance serves to reduce the driving force for Ca\(^{2+}\) extrusion via the Na\(^{+}\)–Ca\(^{2+}\) exchanger and thereby maintains the increase in the intracellular Ca\(^{2+}\) concentration during induction of secretion. Experimental Physiology (2001) 86.4, 461–468.
selectivity of the store-operated conductance in more detail and to determine whether the Na\textsuperscript{+} current mediated by this channel might serve to modify other transporters, i.e. a presumed Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger, involved in intracellular Ca\textsuperscript{2+} homeostasis.

**METHODS**

**Solutions**

For the experiments with isolated crypts the following buffers were used. The EDTA-containing solution for the crypt isolation contained (mmol l\textsuperscript{-1}): NaCl 107, KCl 4.5, NaH\textsubscript{2}PO\textsubscript{4} 0.2, Na\textsubscript{2}HPO\textsubscript{4} 1.8, NaHCO\textsubscript{3} 25, CaCl\textsubscript{2} 10 and glucose 12. The solution was gassed with 5% CO\textsubscript{2}–95% O\textsubscript{2} and kept at a temperature of 37°C, the pH was adjusted to 7.4 with Tris base. The high K\textsuperscript{+} Tyrode solution for storing the crypts consisted of (mmol l\textsuperscript{-1}): potassium gluconate 100, KC\textsubscript{1} 30, NaCl 20, CaCl\textsubscript{2} 1.25, MgCl\textsubscript{2} 1, Hepes 10, glucose 12 and sodium pyruvate 5, with BSA (1 g l\textsuperscript{-1}). The pH was adjusted to 7.4 with KOH.

The superfusion solution during the patch-clamp recordings was a Tyrode solution with the following composition (mmol l\textsuperscript{-1}): NaCl 140, KCl 5.4, Hepes 10, CaCl\textsubscript{2} 1.25, MgCl\textsubscript{2} 1 and glucose 12. To test the permeability of the cation conductance for monovalent cations, NaCl was replaced by an equimolar concentration of CsCl, KCl, LiCl or N-methyl-D-glucamine (NMDG) chloride (NMDGCl). In order to study the modulation of the cation conductance by divergent cations, the following solution was used (mmol l\textsuperscript{-1}): NaCl 140, KCl 5.4, Hepes 10, CaCl\textsubscript{2} 10, MgCl\textsubscript{2} 1 and glucose 12. The CaCl\textsubscript{2} in this buffer was replaced either by BaCl\textsubscript{2}, MgCl\textsubscript{2} or SrCl\textsubscript{2}; 0.5 mmol l\textsuperscript{-1} CaCl\textsubscript{2} was then added to each solution to maintain the integrity of the seals.

In order to reduce contamination by Cl\textsuperscript{-} currents during the whole-cell recordings, a Cl\textsuperscript{-}-free pipette solution was used with a high Ca\textsuperscript{2+}-buffering capacity to activate the store-operated cation conductance. This pipette solution contained (mmol l\textsuperscript{-1}): NMDG gluconate 140, MgCl\textsubscript{2} 2, EGTA 11, CaCl\textsubscript{2} 1, Tris 10, ATP (disodium salt) 5; pH 7.2.

The Ussing chamber experiments were carried out in a Parsons solution containing (mmol l\textsuperscript{-1}): NaCl 107, KCl 4.5, NaHCO\textsubscript{3} 25, Na\textsubscript{2}HPO\textsubscript{4} 1.8, NaH\textsubscript{2}PO\textsubscript{4} 0.2, CaCl\textsubscript{2} 1.25, MgSO\textsubscript{4} 1 and glucose 12. The solution was gassed with 5% CO\textsubscript{2}–95% O\textsubscript{2} and kept at a temperature of 37°C; pH 7.4. For the Cl\textsuperscript{-}-free solution, sodium gluconate replaced NaCl.

**Tissue preparation**

Wistar rats (n = 63) were used with a weight of 120–220 g. The animals had free access to water and food until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination (approved by Regierungspräsidium Gießen, Gießen, Germany). The serosa and muscularis propria were stripped away by hand to obtain the mucosa–submucosa preparation of the colon. The appearance of palm-like striae was used to define the beginning of the proximal colon (Lindström et al. 1979). Two proximal and two distal colonic segments could be prepared from each rat for the Ussing chamber experiments.

**Short-circuit current measurement**

The tissue was fixed in a modified Ussing chamber, bathed with a volume of 3.5 ml on each side of the mucosa and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen, Germany) with correction for solution resistance. The exposed surface of the tissue was 1 cm\textsuperscript{2}. Short-circuit current (I\textsubscript{sc}) was continuously recorded and tissue conductance (G\textsubscript{t}) was measured every minute. The baseline measurements were determined as the mean value during the 3 min just before administration of a drug.

**Crypt isolation**

The mucosa–submucosa was fixed on a plastic holder with tissue adhesive and transferred for 8 min to the EDTA solution. The mucosa was vibrated for 30 s in order to isolate intact crypts. They were collected in an intracellular-like high K\textsuperscript{+} Tyrode solution (Böhme et al. 1991). The mucosa was kept at 37°C during the isolation procedure. All further steps, including the patch clamp and the fura-2 experiments, were carried out at room temperature.

**Patch-clamp experiments**

The crypts were pipetted into the experimental chamber (volume of the chamber 0.5 ml) and fixed to the glass bottom of the chamber with the aid of poly-1-lysine (molecular weight > 300 kDa; 0.1 g l\textsuperscript{-1}). The preparation was superfused hydrostatically throughout the experiment (perfusion rate ~1 ml min\textsuperscript{-1}). The chamber was mounted on the stage of an inverted microscope (Olympus IX-70).

Patch pipettes were pulled from thick-walled borosilicate glass capillaries (Jencons Scientific Ltd, Bedfordshire, UK; o.d., 1.7 mm; i.d., 1.16 mm) on a two-stage puller (H. Ochozki, Homburg/Saar, Germany). After fire-polishing, the tips had resistances of 5–10 MΩ when filled with the standard pipette solution. To obtain a whole-cell recording, the membrane patch under the tip of the pipette was broken by a strong suction pulse after formation of the seal. Opening of the patch was indicated by an increase in the capacitance, a decrease in the resistance and a stable membrane potential under current-clamp conditions. Membrane capacitance was corrected for by cancellation of the capacitance transient (subtraction) using a 50 mV pulse, which was applied every second.

Patch-clamp currents were recorded on an RK-400 amplifier (Biologics, Meylan, France). Current and voltage signals were digitised at 48 kHz and stored on a modified digital audio recorder (DTR-1200, Biologics). The reference point for the patch potentials was the extracellular side of the membrane assumed to have zero potential. All experiments were performed under voltage-clamp conditions; membrane inward current was registered at a holding potential of ~80 mV.

**Fura-2 experiments**

Relative changes in intracellular Ca\textsuperscript{2+} concentration were measured using the Ca\textsuperscript{2+}-sensitive fluorescent dye, fura-2 (Grynkiewicz et al. 1985) as described previously (Frings et al. 1999). The crypts were pipetted into the experimental chamber with a volume of about 3 ml and fixed to the glass bottom of the chamber with the aid of poly-1-lysine (0.1 g l\textsuperscript{-1}). They were loaded for 60 min with 2.5 µmol l\textsuperscript{-1} of the acetoxymethylester form of fura-2 (fura-2 AM) in the presence of 0.05 g l\textsuperscript{-1} Pluronic F-127. Then the fura-2 was washed away. The preparation was superfused hydrostatically throughout the experiment with 140 mmol l\textsuperscript{-1} NaCl Tyrode solution. Perfusion rate was ~1 ml min\textsuperscript{-1}.

Experiments were carried out on an inverted microscope (Olympus IX-50) equipped with an epifluorescence set-up and an image analysis system (Till Photonics, Martinsried, Germany). The emission above 470 nm was measured from several regions of interest, each the approximate size of one cell. The cells were excited alternately at 340 and 380 nm and the ratio of the emission signal at both excitation wavelengths was calculated.
Data were sampled at 0.2 Hz. The baseline fluorescence ratio of fura-2 was measured for several minutes before drugs were administered. An in vitro calibration with extracellular solutions (Molecular Probes, Leiden, The Netherlands) containing free fura-2 and nine different concentrations of free Ca\(^{2+}\) ranging from 0 to 39.8 \(\mu\)mol l\(^{-1}\) was performed in order to estimate the intracellular Ca\(^{2+}\) concentration using the Grynkiewicz equation (Grynkiewicz et al. 1985).

**Drugs**

Fura-2 AM and free fura-2 acid (both from Molecular Probes) were dissolved in dimethylsulfoxide (DMSO; final maximal concentration 2.5 ml l\(^{-1}\)). Pluronic (BASF, Weyandotte, USA) was dissolved in DMSO as a 200 g l\(^{-1}\) stock solution (final maximal DMSO concentration 2.5 ml l\(^{-1}\)). 2',4'-Dichloro-benzamil hydrochloride (Molecular Probes) was dissolved in DMSO (final maximal concentration 2.8 ml l\(^{-1}\)). Tetrodotoxin (Alomone Labs, Jerusalem, Israel) was dissolved as a stock solution in citrate buffer (20 mmol l\(^{-1}\)). Carbachol was dissolved in distilled water. All chemicals were from Sigma, Deisenhofen, Germany, unless otherwise indicated.

**Statistics**

Results are given as means ± one standard error of the mean (S.E.M.). When the means of several groups had to be compared, an analysis of variance was performed followed by Tukey’s post hoc test for comparison of linear contrasts. Comparisons between two groups were carried out using Student’s \(t\) test or the Mann-Whitney \(U\) test. An \(F\) test decided which test method was to be used. Both Student’s paired and unpaired two-tailed \(t\) tests were applied as indicated.

**RESULTS**

Permeability of the store-operated cation conductance for monovalent cations

In a first series of experiments, the permeability of the store-operated cation conductance for different monovalent cations was tested. The store-operated conductance was activated by using a pipette solution with a high Ca\(^{2+}\)-buffering capacity (11 mmol l\(^{-1}\) EGTA–1 mmol l\(^{-1}\) Ca\(^{2+}\)). Contamination by Cl\(^-\) currents, which together with K\(^+\) currents dominate cellular conductance in rat colonic crypt cells under standard conditions (Schultheiss & Diener, 1998), was reduced by the use of NMDG gluconate as the main electrolyte in the pipette solution. The cells were voltage clamped at a membrane potential of −80 mV to drive an inward current, i.e. an influx of cations into the cell (Fig. 1A). The cells were first superfused with a solution containing the large organic cation NMDG\(^{+}\) (140 mmol l\(^{-1}\) NMDGCl Tyrode solution) as the main cation, as it was assumed that the store-operated cation

![Figure 1](image-url)

**Figure 1**

_A_. scheme of the experimental procedure used to determine the permeability of the non-selective, store-operated cation conductance for different monovalent cations (X\(^+\)). The patch pipette contained an NMDG gluconate solution with a high Ca\(^{2+}\)-buffering capacity; the cells were voltage clamped at a membrane potential of −80 mV to drive an inward current. _B_. original record of inward current during superfusion of a crypt with 140 mmol l\(^{-1}\) NMDGCl Tyrode solution (filled bars), and CsCl, LiCl, KCl or NaCl (each 140 mmol l\(^{-1}\); open bars) Tyrode solution, respectively. The different monovalent cations were administered in a random fashion in each experiment. _C_. inward current carried by different monovalent cations tested using the protocol shown in Fig. 1B, normalised to the current flowing in the presence of Na\(^+\), which was set to 100% for each cell. Mean inward current in the presence of Na\(^+\) was −458 ± 220 pA. Values are means ± s.e.m., \(n = 7\); different letters indicate significant differences \((P < 0.05)\) between the individual groups (analysis of variance followed by Tukey’s post hoc test).
conductance is not permeable to NMDG (Fig. 1B). Then the NMDGCl-containing solution was exchanged for one containing an equimolar concentration of CsCl, KCl, LiCl or NaCl in a random sequence. After each change, superfusion was switched back to the NMDGCl solution. All alkali metal ions caused an increase in inward current, i.e. were able to pass across the non-selective cation conductance. The permeability sequence was: Cs\(^+(a) > Na\(^+(b) > Li\(^+(b) (Fig. 1C, n = 7; different letters indicate significant differences at \(P < 0.05\) between the individual groups; analysis of variance followed by Tukey’s post hoc test). The apparent K\(^+\) permeability was not determined because no attempts were made to block cellular K\(^+\) conductances in this series of experiments.

Modulation by different divalent cations
The store-operated conductance is permeable to Na\(^+\) and Ca\(^{2+}\), but in contrast to Na\(^+\), at concentrations of Ca\(^{2+}\) above 1 mmol l\(^{-1}\) the divalent cation exerts a (feedback) inhibition on its own influx (Frings et al. 1999). Using the same NMDG gluconate pipette solution with a high Ca\(^{2+}\)-buffering capacity as described above, the cells were superfused with a Tyrode solution containing 140 mmol l\(^{-1}\) NaCl and a lowered concentration of CaCl\(_2\) (0.5 mmol l\(^{-1}\)). Cells were clamped at −80 mV and membrane inward current, carried mainly by an influx of Na\(^+\) under these conditions (Frings et al. 1999), was monitored (Fig. 2A). Then 10 mmol l\(^{-1}\) BaCl\(_2\), CaCl\(_2\), MgCl\(_2\) or SrCl\(_2\) were added in a random sequence to the superfusion medium and the resulting decrease in inward current was recorded (Fig. 2B). After each exchange, cells were superfused again with the ‘standard’ perfusion solution as illustrated in Fig. 2B. All divalent cations reduced the Na\(^+\) inward current. Their apparent efficiency to inhibit the cation conductance was: Ca\(^{2+}(a) > Mg^{2+}(a,b) > Ba^{2+}(b,c) > Sr^{2+}(c) (Fig. 2C, n = 7; different letters indicate significant differences at \(P < 0.05\) between the individual groups; analysis of variance followed by Tukey’s post hoc test). In general, there was a run-down of the inward current during these experiments with the transient elevation of the extracellular concentration of divalent cations (Fig. 2B). Therefore the sequence of the solution changes was varied in a random fashion in each of the seven experiments in order to avoid a systematic error due to this run-down. Nevertheless the experiments clearly show that Ca\(^{2+}\) and Mg\(^{2+}\), the only physiologically important divalent cations, exert the same level of inhibition.

**Figure 2**

*A*, scheme of the experimental procedure used to determine the inhibitory effect of divalent cations (X\(^{2+}\)) on the non-selective, store-operated cation conductance. The patch pipette contained an NMDG gluconate solution with a high Ca\(^{2+}\)-buffering capacity; the cells were voltage clamped at a membrane potential of −80 mV to drive an inward current carried mainly by Na\(^+\). *B*, original record of inward current during superfusion of a crypt with 140 mmol l\(^{-1}\) NaCl, 0.5 mmol l\(^{-1}\) CaCl\(_2\) Tyrode solution (open bar), to which 10 mmol l\(^{-1}\) CaCl\(_2\), BaCl\(_2\), SrCl\(_2\) or MgCl\(_2\) (filled bars), respectively, were added. The different divalent cations were administered in a random fashion in each experiment. *C*, inhibition of inward Na\(^+\) current by different divalent cations using the protocol shown in Fig. 1B. Data were normalised to the inhibition produced by 10 mmol l\(^{-1}\) CaCl\(_2\), which was set to 100% for each cell. Mean reduction of inward current by 10 mmol l\(^{-1}\) Ca\(^{2+}\) was 166 ± 63 pA. Values are means ± s.e.m., n = 7; different letters indicate significant differences (\(P < 0.05\)) between the individual groups (analysis of variance followed by Tukey’s post hoc test).
Functional demonstration of the presence of a Na⁺–Ca²⁺ exchanger

As pointed out in the Introduction, the dominant Na⁺ influx via the store-operated cation conductance, which is the physiological response of the colonocytes to an increase in intracellular Ca²⁺ concentration, does not induce anion secretion. However, for example in the heart it is known that Na⁺ influx and depolarisation that occur during the action potential can turn off or even reverse the Na⁺–Ca²⁺ exchanger, which will thus transport Ca²⁺ into the cell under these conditions (for review see Blaustein & Lederer, 1999). Consequently, we tried to determine whether colonic enterocytes express a functional Na⁺–Ca²⁺ exchanger. For this purpose, crypts were loaded with the Ca²⁺-sensitive dye fura-2, to investigate whether the cells respond to a reversal of the normal Na⁺ gradient with a change in their intracellular Ca²⁺ concentration.

When the superfusion medium was exchanged from Tyrode solution (140 mmol l⁻¹ NaCl) to a Na⁺-free Tyrode solution (140 mmol l⁻¹ NMDGCl), a prompt increase in the fluorescence ratio of fura-2 was observed, indicating an increase in the intracellular Ca²⁺ concentration (Fig. 3A). The response started after a delay of a few minutes. Five minutes after administration of NMDG⁺, the fura-2 ratio had increased from 1.5 ± 0.1 to 2.1 ± 0.2 (n = 6; P < 0.05, paired t test). This corresponds to an increase in the intracellular Ca²⁺ concentration from 260 ± 39 to 436 ± 60 nmol l⁻¹ (n = 6; P < 0.05, paired t test) when using an extracellular calibration to estimate the changes in the intracellular Ca²⁺ from the measured fluorescence ratios. Because extracellular calibration does not consider possible interactions of fura-2 with intracellular components (Baylor & Hollingworth, 2000), the absolute values of the intracellular Ca²⁺ concentration have to be considered with caution. Nevertheless these data suggest the presence of a Na⁺–Ca²⁺ exchanger, which under control conditions, i.e. during superfusion with a Na⁺-containing solution, is used to extrude Ca²⁺ from the cell, and which is reversed in its direction in the Na⁺-free buffer.

In order to test this assumption, the response to the Na⁺-free Tyrode solution (140 mmol l⁻¹ NMDGCl) was measured in the presence of dichlorobenzamil (2.5 µmol l⁻¹), an inhibitor of Na⁺–Ca²⁺ exchangers (Kaczorowski et al. 1985). The amiloride analogue, dichlorobenzamil itself caused an apparent decrease in the fluorescence ratio (Fig. 3B). This effect has already been observed for the mother compound of dichlorobenzamil, benzylamiloride, and has been attributed to a quenching of the fura-2 signal by this aromatic substance (Hudson et al. 1998). Switching the superfusion to the 140 mmol l⁻¹ NMDGCl Tyrode solution in the presence of dichlorobenzamil no longer evoked any increase in the ratio signal (n = 8; P < 0.05 vs. response in the absence of dichlorobenzamil, unpaired t test), indicating that the increase in the intracellular Ca²⁺ concentration is indeed caused by the activity of the Na⁺–Ca²⁺ exchanger working as a Ca²⁺ influx pathway under Na⁺-free conditions.

Intact mucosa

Because dichlorobenzamil is able to the quench the fura-2 signal (Hudson et al. 1998), it could not be used in fura-2 experiments to investigate whether the Na⁺–Ca²⁺ exchanger functions normally as a Ca²⁺-extruding carrier. Therefore, we used a different approach to address this question. Dichlorobenzamil (100 µmol l⁻¹ at the mucosal and the serosal side)
was administered to mucosa–submucosa preparations of rat colon. In the proximal colon, the drug consistently induced an increase in \( I_{sc} \) from 2.1 ± 0.3 to 2.9 ± 0.3 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \) in all nine tissues tested (\( P < 0.05 \), paired \( t \) test). This response was insensitive to the \( \text{Na}^+ \) channel blocker, tetrodotoxin (TTX). In the presence of TTX (1 \( \mu \text{mol} \text{l}^{-1} \) at the serosal side) dichlorobenzamil induced an increase in \( I_{sc} \) of 0.9 ± 0.3 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \) (\( n = 5 \)), which was not significantly different from the \( I_{sc} \) response in the absence of the blocker (0.7 ± 0.1 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \); see above). In contrast, in the distal colon, the effect of dichlorobenzamil was inconsistent. In 8 out of 14 tissues, \( I_{sc} \) increased from 1.5 ± 0.3 to 2.4 ± 0.4 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \) (\( P < 0.05 \), paired \( t \) test; Fig. 4A), whereas 6 out of 14 tissues responded with a decrease in \( I_{sc} \) from 2.3 ± 0.3 to 1.5 ± 0.3 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \) (\( P < 0.05 \), paired \( t \) test). The effect of dichlorobenzamil showed a dependence on the \( I_{sc} \) prior to administration of the drug (Fig. 4C), i.e. in tissues with a low baseline \( I_{sc} \) dichlorobenzamil induced an increase in \( I_{sc} \), whereas in tissues with a high baseline \( I_{sc} \) the drug evoked a decrease in \( I_{sc} \). When the distal colonic tissues were treated with TTX (1 \( \mu \text{mol} \text{l}^{-1} \) at the serosal side), \( I_{sc} \) decreased by 0.6 ± 0.2 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \) (\( n = 6 \), \( P < 0.05 \), paired \( t \) test). Under these conditions, all tissues tested (\( n = 6 \)) responded with an increase in \( I_{sc} \) after administration of dichlorobenzamil, which amounted to 0.6 ± 0.1 \( \mu \text{equiv h}^{-1} \text{cm}^{-2} \) (\( n = 6 \)). All changes in \( I_{sc} \) were completely suppressed in both colonic segments, when the dichlorobenzamil was applied in the absence of \( \text{Cl}^- \) (\( n = 8 \); Fig. 4B).

In a final set of experiments we investigated the mode of action of the exchanger during activated \( \text{Ca}^{2+} \)-dependent anion secretion. We used carbachol, the stable cholinergic agonist which stimulates muscarinic M3 receptors at the colonic epithelium (Lindqvist et al. 1998), as a \( \text{Ca}^{2+} \)-dependent secretagogue. As described previously (Strabel & Diener, 1995), carbachol (50 \( \mu \text{mol} \text{l}^{-1} \) at the serosal side) causes a fast increase in \( I_{sc} \) with a subsequent decay, which follows a bi-exponential function with a fast and a slow time constant. When dichlorobenzamil (100 \( \mu \text{mol} \text{l}^{-1} \) at the mucosal and the serosal side) was administered during the decaying phase of the carbachol response, the drug no longer

\[ \Delta I_{sc} (\mu \text{Eq h}^{-1} \text{cm}^{-2}) \]

\[ \text{Baseline } I_{sc} (\mu \text{Eq h}^{-1} \text{cm}^{-2}) \]

Figure 4

\( A \), original record demonstrating the effect of dichlorobenzamil (100 \( \mu \text{mol} \text{l}^{-1} \) at the mucosal and the serosal side; filled bar) on \( I_{sc} \) in the presence of \( \text{Cl}^- \) ions (107 \( \text{mmol l}^{-1} \) \( 
\text{NaCl} \) Parsons solution at both sides of the tissue; open bar). The tracing is typical for all 9 experiments at the proximal, and 8 out of 14 experiments at the distal, colon; for statistics, see text. \( B \), original record demonstrating the lack of effect of dichlorobenzamil (100 \( \mu \text{mol} \text{l}^{-1} \) at the mucosal and the serosal side; filled bar) on \( I_{sc} \) in the absence of \( \text{Cl}^- \) ions (107 \( \text{mmol l}^{-1} \) sodium gluconate Parsons solution at both sides of the tissue; open bar). The tracing is typical for 8 experiments each at the proximal and distal colon. \( C \), dependence of the dichlorobenzamil effect (\( \Delta I_{sc} \)) on the baseline \( I_{sc} \) in the distal colon: increase of \( I_{sc} \) at lower baselines in 8 out of 14 tissues (filled square, mean ± S.E.M.) and decrease at higher baselines in 6 out of 14 tissues (open square, mean ± S.E.M.).

Figure 5

Original record demonstrating the effect of dichlorobenzamil (100 \( \mu \text{mol} \text{l}^{-1} \) at the mucosal and the serosal side; filled bar) on \( I_{sc} \) in rat colon in the presence of carbachol (50 \( \mu \text{mol} \text{l}^{-1} \) at the serosal side; open bar). The tracing is typical for 5–7 experiments at the proximal and the distal colon.
caused an increase in $I_e$ in either colonic segment (Fig. 5), indicating that in contrast to basal conditions, the Na$^+$–Ca$^{2+}$ exchanger no longer extrudes Ca$^{2+}$ when the store-operated non-selective cation conductance is activated by carbamol. In the presence of dichlorobenzamil, $I_e$ decreased by $0.5 \pm 0.2 \mu$equiv h$^{-1}$ cm$^{-2}$ ($n = 7$) in the distal and $0.4 \pm 0.2 \mu$equiv h$^{-1}$ cm$^{-2}$ ($n = 7$) in the proximal colon within 2 min of administration of the blocker. This response was not different from the spontaneous decrease in $I_e$ measured in time-dependent control experiments, where $I_e$ decreased by $0.5 \pm 0.1 \mu$equiv h$^{-1}$ cm$^{-2}$ ($n = 7$) in the distal and $0.3 \pm 0.1 \mu$equiv h$^{-1}$ cm$^{-2}$ ($n = 5$) in the proximal colon within the same period of time.

DISCUSSION

Colonic enterocytes respond to an increase in the intracellular Ca$^{2+}$ concentration with a secretion of Cl$^-$. In intact mucosa, this Cl$^-$ secretion is an indirect response caused by an increase in the driving force for Cl$^-$ exit via apical Cl$^-$ channels, i.e. the cystic fibrosis transmembrane conductance regulator (CFTR) channels, as Ca$^{2+}$-sensitive basolateral K$^+$ channels are opened (Böhme et al. 1991; Strabel & Diener, 1995; Bleich et al. 1996). In undifferentiated colonic tumour cell lines (Anderson & Walsh, 1991) and after treatment of the mucosa with carcinogens (Bleich et al. 1997) a second site of action of intracellular Ca$^{2+}$ develops, i.e. Ca$^{2+}$-dependent apical Cl$^-$ channels, which are expressed under these conditions. A classical agonist that induces Ca$^{2+}$-mediated anion secretion is acetylcholine which, via muscarinic M$_3$ receptors, stimulates phospholipase C and induces the release of Ca$^{2+}$ from intracellular inositol-1,4,5-trisphosphate-sensitive stores (Goyal, 1989; O’Malley et al. 1995; Lindqvist et al. 1998). This initial Ca$^{2+}$ release is followed by an influx of Ca$^{2+}$ from the extracellular medium due to the opening of store-operated ion channels in the cell membrane (for review see Parekh & Penner, 1997). The exact mode of coupling between the intracellular stores and the ion channels in the membrane is not yet known; there are data arguing both for a direct mechanical interaction (see e.g. Yao et al. 1999) as well as for the involvement of a soluble messenger substance (see e.g. Trepakova et al. 2000). The molecular identity of the store-operated cation channels is currently under debate; there is evidence for a role of members of the trp-channel family in the mediation of store-operated cation influx (Philipp et al. 1996, 1998).

In rat colon, store depletion activates a non-selective cation conductance (Frings et al. 1999). The permeability sequence for monovalent cations of this conductance is: Cs$^+ >$ Na$^+$ ≥ Li$^+$ (Fig. 1). Cs$^+$ possesses the largest ionic diameter with the consequence that its dehydration energy is smaller compared to that of Na$^+$ or Li$^+$ (Hille, 1991). This suggests that the monovalent cations have to be dehydrated before they can pass across the non-selective channel. In general, the inward current in the presence of extracellular KCl had a similar amplitude to that measured in the presence of extracellular CsCl (Fig. 1B). However, no attempts were made to determine the apparent K$^+$ permeability of the non-selective cation conductance because of the presence of additional cellular K$^+$ conductances. Nevertheless the underlying channels will allow a dominant influx of Na$^+$ under physiological conditions, i.e. a high Na$^+$ and a low K$^+$ concentration in the extracellular space.

As shown previously (Frings et al. 1999), the store-operated cation conductance exhibits a complex interaction with Ca$^{2+}$. This conductance is permeable to Ca$^{2+}$, but in addition Ca$^{2+}$ exerts a (feedback) inhibition on its own influx at concentrations exceeding 1 mmol l$^{-1}$. The present experiments demonstrate that other divalent cations share this inhibitory action with the sequence: Ca$^{2+} ≥$ Mg$^{2+} ≥$ Ba$^{2+} ≥$ Sr$^{2+}$ (Fig. 2). We can only speculate from our whole-cell data about the mechanism of the inhibitory action of divalent cations: they might exert their inhibitory action by binding to an extracellular or an intracellular site or they might even bind within the pore structure of the channel. Obviously, this putative binding site can only discriminate poorly between different divalent cations. This inhibition by divalent cations is probably responsible for the ‘run-down’ of the cation inward current in the experimental series with the elevated concentration of divalent cations. Consequently, Ca$^{2+}$, passing into the cell via the store-operated cation conductance, limits its own influx, which might protect the cell from an excessive increase in the cytoplasmic Ca$^{2+}$ concentration.

In rat arterial smooth muscle cells it has been shown that Na$^+$ entry via store-operated cation channels influences other transporters such as a Na$^+$–Ca$^{2+}$ exchanger (Arnon et al. 2000). Consequently, we investigated whether such an exchanger might be present in colonic epithelium and whether it might interact with the store-operated cation conductance. The functional expression of the exchanger was demonstrated in cation substitution experiments using crypts loaded with fura-2. Replacing the extracellular Na$^+$ with an impermeant cation, NMDG$^+$, which will reverse the normal, inward gradient for Na$^+$ into an outward gradient, induced an increase in the fluorescence ratio of fura-2 indicating an increase in the intracellular Ca$^{2+}$ concentration (Fig. 3). This response was suppressed in the presence of dichlorobenzamil, which has been shown to act as a blocker of Na$^+$–Ca$^{2+}$ exchangers (Kaczorowski et al. 1985), although this observation is complicated by the quenching properties of this blocker (Hudson et al. 1998). Consequently, during superfusion with NMDG$^+$, the Na$^+$–Ca$^{2+}$ exchanger acts as a Ca$^{2+}$-loading transporter, as the outward electrochemical gradient for Na$^+$ exceeds the gradient for Ca$^{2+}$.

Under physiological conditions, however, the exchanger seems to work as a Ca$^{2+}$-extruding pathway. Because of the fluorescence properties of dichlorobenzamil, the effect of inhibition of the exchanger on basal Ca$^{2+}$ concentration could not be directly determined in fura-2 experiments. Therefore, we used the indirect approach of investigating the response to this inhibitor of the colonic mucosa mounted in the Ussing chamber. Dichlorobenzamil induced a prompt increase in $I_e$ in most tissues tested (9 out of 9 tissues from the proximal colon and 8 out of 14 from the distal colon), a response which was completely dependent on the presence of Cl$^-$ ions and insensitive to the Na$^+$ channel blocker TTX, indicating that
the drug stimulates a Cl− secretion directly at the epithelial cells (Fig. 4). The effect of dichlorobenzamil showed a dependence on baseline $I_{sc}$ (Fig. 4C), i.e. in tissues with a low baseline $I_{sc}$ dichlorobenzamil induced an increase in $I_{sc}$, whereas in tissues with a high baseline $I_{sc}$ it induced a decrease in $I_{sc}$. This action is consistent with the idea that under basal conditions in tissues with a low baseline $I_{sc}$, the Na+/Ca2+ exchanger extrudes Ca2+ out of the cells, so that inhibition of the exchanger will cause an increase in the intracellular Ca2+ concentration thereby inducing secretion of Cl−. This idea is supported by the observation that in the presence of TTX, i.e. under conditions, where the baseline $I_{sc}$ is lowered, dichlorobenzamil always induced an increase in $I_{sc}$. It is interesting to note that after stimulation of muscarinic receptors with carbachol, i.e. after inositol-1,4,5-trisphosphate-induced store depletion and consequent activation of the exchanger, the Na+/Ca2+ exchanger is turned off.

Consequently, the high Na+ permeability of the store-operated cation conductance in rat colonic epithelium may serve as an inhibitory signal for a Ca2+-extruding pathway, i.e. the Na+/Ca2+ exchanger, and thereby indirectly contribute to the maintenance of an elevated intracellular Ca2+ concentration.

References


BINDER, H. J. & SANDLE, G. J. (1994). Electrolyte transport in the exchanger will cause an increase in the intracellular Ca2+. Induced store depletion and consequent activation of the receptors with carbachol, i.e. after inositol-1,4,5-trisphosphate-interesting to note that after stimulation of muscarinic FRINGS, M., SCHULTHEISS, G. & DIENER, M. (1999). Electrogenic cation conductance in rat colonic epithelium may serve as an inhibitory signal for a Ca2+-extruding pathway, i.e. the Na+/Ca2+ exchanger extrudes Ca2+ out of the cells, so that inhibition of the exchanger extrudes Ca2+ out of the cells, so that inhibition of the exchanger will cause an increase in the intracellular Ca2+ concentration thereby inducing secretion of Cl−. This idea is supported by the observation that in the presence of TTX, i.e. under conditions, where the baseline $I_{sc}$ is lowered, dichlorobenzamil always induced an increase in $I_{sc}$. It is interesting to note that after stimulation of muscarinic receptors with carbachol, i.e. after inositol-1,4,5-trisphosphate-induced store depletion and consequent activation of the exchanger, the Na+/Ca2+ exchanger is turned off.

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