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ELECTROLYTE TRANSPORT THROUGH A CATION-SELECTIVE ION CHANNEL IN LARGE INTESTINAL ENTEROCYTES OF *XENOPUS LAEVIS*

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Summary

Electrogenic ion transport through the colon epithelium of the African clawed toad (*Xenopus laevis*) was investigated with electrophysiological methods *in vitro*. Interest was focused on a previously described phenomenon, that removal of Ca^{2+} from the mucosal Ringer's solution increases electrogenic sodium absorption. Our results clearly show that Ca^{2+} removal reveals an apical ion channel that is not a specific Na^+ channel, but a non-selective cation channel with an 'apparent' ion selectivity of the order $K^+ > Na^+ = Rb^+ > Cs^+ > Li^+$. This Ca^{2+}-sensitive current increased linearly with the mucosal pH, and could be inhibited by other divalent cations (Mg^{2+}, Ba^{2+}) and the organic ion channel blockers quinidine and verapamil. The mucosal Ca^{2+} concentration that induced a half-maximal inhibition of the Ca^{2+}-sensitive current was about 1 μmol l^{-1} and was independent of the mucosal pH. Owing to the high Ca^{2+} sensitivity, a regulation of the channel conductivity by extracellular Ca^{2+} is ruled out. It is concluded that this channel, which is almost identical to similar channels found in amphibian skin and bladder, acts as a pathway for cation absorbing or secreting processes. Possibly the binding of extracellular Ca^{2+} is related to selectivity changes of the Ca^{2+}-sensitive ion channel.

Introduction

In numerous vertebrates, much of the transepithelial electrical potential difference across the large intestine is generated by electrogenic Na^+ absorption (Groot and Bakker, 1988), so that sodium ions diffuse into the epithelial cell through specific apical Na^+ channels, which are highly sensitive to the epithelial Na^+ channel blocker amiloride (Benos, 1986). The sodium ions are then extruded to the interstitium by the basolateral Na^+/K^+-ATPase. In amphibians, this transport has been intensively studied *in vitro* in both toads (*Bufo* sp.) (Cofré and Crabbé, 1967; Dawson and Curran, 1976) and frogs (*Rana* sp.) (Krattenmacher

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and Clauss, 1988, 1989; Krattenmacher *et al.* 1988). Electrogenic Na^+ transport in the colon of these amphibians follows the general model mentioned above. However, in the African clawed toad (*Xenopus laevis*) the apical entry step differs considerably from this model. A previous study (Krattenmacher *et al.* 1990) demonstrated that, in *Xenopus*, Na^+ passes the apical membrane of the colonic epithelial cells through an amiloride-insensitive ion channel. Furthermore, the amount of Na^+ absorption is increased by about 120 % in the absence of mucosal calcium. Thus, electrogenic Na^+ absorption in the amphibian colon does not follow a general and uniform mechanism.

In intestinal epithelia a similar Ca^{2+} sensitivity has only been reported in the rumen of sheep (Martens *et al.* 1990) and it has not been described in the large intestine. In non-intestinal epithelia it was described in the skin of larval (Hillyard *et al.* 1982) and adult frogs (Van Driessche and Zeiske, 1985) and in toad skin (Aelvoet *et al.* 1988) and urinary bladder (Van Driessche, 1987; Van Driessche *et al.* 1987; Aelvoet *et al.* 1988; Das and Palmer, 1989). In these amphibian tissues, micromolar concentrations of 'mucosal' calcium blocked this channel, which, in the absence of mucosal calcium, was also permeable to other monovalent cations. Van Driessche (1987) speculated that this channel might represent an epithelial Ca^{2+} channel, because similar properties have been described for Ca^{2+} channels in frog muscle fibres (Almers *et al.* 1984; Almers and McCleskey, 1984; Hess *et al.* 1986). A recent patch-clamp study by Das and Palmer (1989) indicated that the channel seems to be a non-selective cation channel that may be involved in Na^+-absorbing or K^+-secreting processes. However, the physiological function of this Ca^{2+}-sensitive channel is unclear. In contrast, in the colon of *Xenopus*, Krattenmacher *et al.* (1990) demonstrated that the Ca^{2+}-sensitive pathway plays a significant role in electrogenic Na^+ absorption, even in the presence of mucosal calcium. In this study we have looked at the physiological role of this channel by investigating the Ca^{2+}-sensitive pathway in the colon of *Xenopus* in more detail.

Materials and methods

Animals and tissue preparation

Breeding adult frogs (*Xenopus laevis*; Horst Kähler, Hamburg, FRG) of either sex were used for the experiments. They were kept at room temperature in tap water and fed once a week with commercial cat food (Brekkies, EFFEM GmbH Verden, FRG). The frogs were pithed, the colon was quickly removed, opened along the mesenteric border and rinsed with frog Ringer's solution. The tissue was stretched and glued (Histoacryl Blue, Braun Melsungen, FRG) serosal side downwards onto a Lucite ring. The inner diameter of the ring was 8 mm. The preparations were mounted in an Ussing chamber which was specially designed to avoid edge damage (De Wolf and Van Driessche, 1986). Silicone grease (Bayer Silicone) was used to seal the edges on both sides of the mounted tissue. Effective tissue area was 0.5 cm^2. The mucosal and serosal chamber compartments were

continuously perfused with frog Ringer's solution (room temperature) at a flow rate of about 5 ml min^{-1}.

Electrical measurements

The spontaneous transepithelial potential difference was clamped to zero with a voltage-clamp unit (Elke Nagel, Biomedical Instruments, München, FRG). The resulting short-circuit current (I_{sc}) and the transepithelial conductance (G_T) were continuously recorded by a standard stripchart recorder. G_T was calculated by the voltage-clamp unit from deflections in transepithelial voltage and current, which were induced by superimposed voltage pulses of 10 mV amplitude and 200 ms duration. Pulse frequency was 0.5 Hz. Pulse polarity had no effect on G_T values.

Solutions

The serosal side of the tissue was always exposed to a NaCl–Ringer's solution with the following composition (in mmol l^{-1}): Na^+, 115; K^+, 2.5; Ca^{2+}, 1.0; Cl^-, 117; HCO_3^-, 2.5 and Hepes buffer, 8 {pH 8.0, adjusted with Trizma base [Tris-(hydroxymethyl)-aminomethane]}. The normal mucosal Ringer's solution was of the same composition. However, for selectivity experiments, the main cation Na^+ was substituted by equimolar amounts of Li^+, K^+, Rb^+ or Cs^+, added as chloride salts. For Ca^{2+}-free Ringer's solutions 1 mmol l^{-1} $CaCl_2$ was omitted and 0.5 mmol l^{-1} EGTA was added. In experiments where pH dependency was investigated, the pH of the mucosal Ca^{2+}-free Ringer's solutions (6, 6.5, 7, 7.5 and 8) was adjusted with Trizma base. According to Portzehl *et al.* (1964), free (ionized) calcium concentrations ($[Ca^{2+}]_{free}$) were controlled in the micro- and submicromolar range by utilizing the buffering ligands EDTA (at pH 6.5) and HEEDTA (*N*-hydroxyethylethylenediamine-triacetic acid, at pH 8.0). These ligands have four negative charges (L^{4-}) which may bind H^+ and/or Ca^{2+}. Only the ligands with four (L^{4-}) and three (HL^{3-}) negative charges were considered in the calculations concerning Ca^{2+} binding because the affinity of Ca^{2+} for the ligands H_2L^{2-} and H_3L^- can be neglected. The true association constants of Ca^{2+} for the ligands L^{4-} and HL^{3-} were designated as K_{CaL} and K_{CaHL}.

Because these constants are, in practice, strongly influenced by pH, apparent association constants (K^a_{CaL} and K^a_{CaHL}) were calculated as:

$$K^a_{CaL} = K_{CaL}/(1 + [H^+]K_1 + [H^+]^2K_1K_2 + [H^+]^3K_1K_2K_3 + [H^+]^4K_1K_2K_3K_4) \quad (1)$$

and

$$K^a_{CaHL} = K_{CaHL}/\{1 + 1/([H^+]K_1) + [H^+]K_2 + [H^+]^2K_2K_3 + [H^+]^3K_2K_3K_4\}\,, \quad (2)$$

where K_1, K_2, K_3 and K_4 are the association constants of H^+ to the ligands. $[H^+]$ represents the proton concentration in the solution. K_{CaL}, K_{CaHL}, K_1, K_2, K_3 and K_4 for an ionic strength of 0.1 mol l^{-1} KCl and a temperature of 20 °C were derived

according to Martell and Smith (1974). The equilibrium of Ca^{2+} and the buffering ligand at a given pH can therefore be described by the equation:

$$K^{a}_{CaL} + K^{a}_{CaHL} = [Ca^{2+}]_{bound}/([Ca^{2+}]_{free}[ligand]_{free}) . \quad (3)$$

Because almost all calcium ions are bound to the ligand, $[Ca^{2+}]_{bound}$ can be regarded as the total Ca^{2+} concentration ($[Ca^{2+}]_{tot}$) of the solution. Therefore $[ligand]_{free}$ represents the difference between the total concentration of the buffering ligand ($[ligand]_{tot}$) and $[Ca^{2+}]_{tot}$. Thus, a rearrangement of equation 3 allows the calculation of the free Ca^{2+} concentration in the solution:

$$[Ca^{2+}]_{free} = [Ca^{2+}]_{tot}/\{([ligand]_{tot} - [Ca^{2+}]_{tot})(K^{a}_{CaL} + K^{a}_{CaHL})\} . \quad (4)$$

Chemicals

Quinidine was dissolved in dimethyl sulphoxide (DMSO) and added to the mucosal solution at a final concentration of 0.1–3.2 mmol l^{-1}. Verapamil was dissolved in water and used in the mucosal solution in the concentration range 25–500 μmol l^{-1}. The solubility of verapamil was facilitated by adding about 10 μmol l^{-1} Tween 80 [polyoxyethylen-(20)-sorbitanmono-oleate]. Tween 80 was without effect on short-circuit current. All chemical compounds, except standard salts (Fluka, Buchs, CH), were obtained from Sigma (München, FRG).

Analysis and statistics

Dose–response relationships of the Ca^{2+}-inhibitable short-circuit current (I_{sc}) were analyzed with the direct linear plot method of Eisenthal and Cornish-Bowden (1974). After rearrangement of the Michaelis–Menten equation, the inhibition of the short-circuit current (ΔI_{sc}) by defined concentrations of mucosal free Ca^{2+} ($[Ca^{2+}]_m$) follows the equation:

$$(I^{Ca,max}_{sc}/\Delta I_{sc}) - (K^{Ca}_{m}/[Ca^{2+}]_m) = 1 , \quad (5)$$

where $I^{Ca,max}_{sc}$ is the maximal Ca^{2+}-sensitive short-circuit current and K^{Ca}_{m} is the Michaelis constant. In the case of Michaelis–Menten kinetics, the connection of each data pair of $[Ca^{2+}]_m$ (plotted on the abscissa) and the corresponding ΔI_{sc} (plotted on the ordinate) gives a straight line in the direct linear plot (see Fig. 4). Perfect Michaelis–Menten kinetics would reveal one intersection point for all the straight lines, giving the estimated value of $I^{Ca,max}_{sc}$ (ordinate) and K^{Ca}_{m} (abscissa). However, in practice, the straight lines intersect at several points. According to Eisenthal and Cornish-Bowden (1974), the medians of these intersection values should be used as best estimates of $I^{Ca,max}_{sc}$ and K^{Ca}_{m}. The analysis of the blocking kinetics of quinidine and verapamil was performed using the same procedure.

Results are expressed as mean±standard error of the mean (S.E.M.). N designates the number of experiments. Statistical analysis was done using Student's t-test (if possible for paired observations) with a significance level of $P \leq 0.05$.

Results

After about 30–45 min of equilibration of the voltage-clamped tissue in Ca^{2+}-containing (1 mmol l^{-1}) NaCl–Ringer's solution (pH 8.0), short-circuit current (I_{sc}) and transepithelial conductance (G_T) reached stable values (Table 1). When Ca^{2+} was removed from the mucosal solution after the equilibration time, I_{sc} increased significantly by about 80%. This increase was accompanied by a significant increase (about 70%) in G_T. Both effects were fully reversible on the re-addition of 1 mmol l^{-1} Ca^{2+}. This phenomenon has already been described by Krattenmacher *et al.* (1990), who demonstrated that the current increase is fully explained by a stimulation in electrogenic Na^+ transport from the mucosal to the serosal side of the epithelium. Interestingly, the amount of this Ca^{2+}-sensitive stimulation is strongly dependent on the mucosal pH. Experiments in which the pH of the mucosal Ca^{2+}-free solution was increased in steps (6.0, 6.5, 7.0, 7.5, 8.0) showed a similar stepwise increase in I_{sc} (Fig. 1). A plot of the Ca^{2+}-sensitive current (I_{sc}^{Ca}) *versus* the mucosal pH revealed a strong linear relationship (Fig. 2). Because this effect of pH had already been observed in preliminary experiments,

Table 1. *Short-circuit current (I_{sc}) and transepithelial conductance (G_T) in the presence (control) and absence of mucosal Ca^{2+}*

	Control	Ca^{2+}-free
I_{sc} (μA cm^{-2})	14.6±1.4 (21)	26.3±3.6* (13)
G_T (mS cm^{-2})	1.56±0.28 (21)	2.63±0.47* (13)

Values are given as mean±S.E.M. (N).
* Significantly different from control values ($P \leq 0.05$).

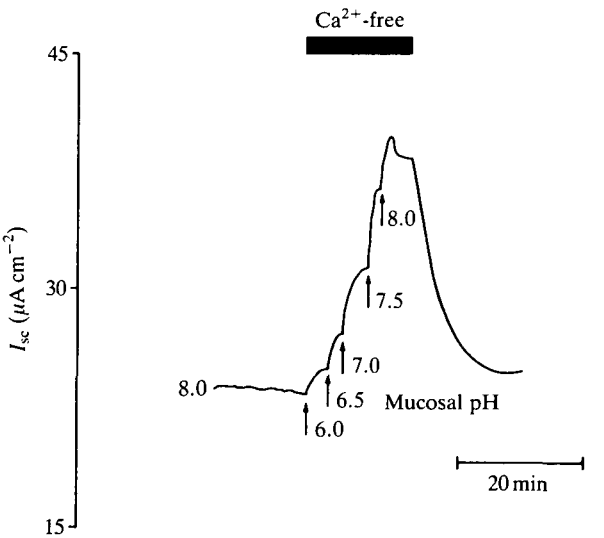

Fig. 1. Changes in the short-circuit current (I_{sc}) during stepwise increases of mucosal pH in the absence of mucosal calcium.

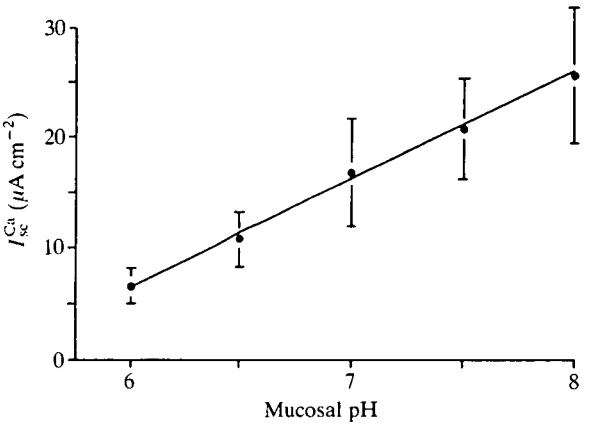

Fig. 2. The dependence of the Ca^{2+}-sensitive short-circuit current (I_{sc}^{Ca}) on the pH of the mucosal Ringer's solution. Values are given as mean±S.E.M. (N=4).

all solutions used in this study contained 8 mmol l^{-1} Hepes buffer to achieve constant pH values. Although in some experiments pH also had a small effect on I_{sc}, even in the presence of mucosal calcium, a clear pH dependency of the Ca^{2+}-insensitive current was not found. Thus, the pH-induced current changes were regarded as changes in Ca^{2+}-sensitive current.

For further characterization of the Ca^{2+}-sensitive current, we blocked the stimulated short-circuit current in a dose-dependent manner by increasing mucosal Ca^{2+}-concentrations. The time course of changes in the I_{sc} in such an experiment is shown in Fig. 3. Accurate micromolar Ca^{2+}-concentrations were obtained using the Ca^{2+}-buffers EDTA (at pH 6.5) and HEEDTA (at pH 8) (for details see Materials and methods). Analysis of the dose–response curves using the direct linear plot (DLP) showed that the stepwise inhibition of the I_{sc} follows Michaelis–Menten-type kinetics (Fig. 4). Using this method we were able to calculate the Michaelis constant of the Ca^{2+}-induced inhibition (K_m^{Ca}). Because of the pH dependency demonstrated in Fig. 2, we calculated K_m^{Ca} at pH values of 6.5 and 8.0. The results of this analysis are given in Table 2. The Michalis constant was less than 1 μmol l^{-1} at both pH values and there was no significant difference between the two. The direct linear plot method gives a calculated value for maximal I_{sc}^{Ca}. Because this calculated value [indicated by I_{sc}^{Ca} (DLP) in Table 2] is similar to the measured value of I_{sc}^{Ca} [indicated by I_{sc}^{Ca} (measured)], the direct linear plot method can be regarded as an appropriate method for calculating K_m^{Ca} in this study. Although I_{sc}^{Ca} clearly increases with increasing mucosal pH, as shown in Fig. 2, the difference between I_{sc}^{Ca} at pH 6.5 and that at pH 8.0 in Table 2 is not significant. This might be explained by the small number of experiments and the wide scatter of the values, at least at pH 8.0.

To investigate whether divalent cations other than Ca^{2+} can block this channel, we substituted all mucosal Ca^{2+} with Mg^{2+}. Fig. 5 shows that the short-circuit current did not change when the mucosal Ringer's solution was changed from

Table 2. *Ca^{2+}-sensitive short-circuit current (I_{sc}^{Ca}) and the Michaelis constant (K_m^{Ca}) of the Ca^{2+} dose–response curve at different values of mucosal pH*

	pH 6.5	pH 8.0
I_{sc}^{Ca} (measured) ($\mu A\,cm^{-2}$)	3.0±0.8 (3)	6.5±2.4 (4)
I_{sc}^{Ca} (DLP) ($\mu A\,cm^{-2}$)	2.9±0.3 (3)	5.5±1.5 (4)
K_m^{Ca} (DLP) ($\mu mol\,l^{-1}$)	0.84±0.35 (3)	0.97±0.32 (4)

I_{sc}^{Ca} (measured) is the measured difference in short-circuit current induced by removal of mucosal Ca^{2+}, whereas DLP indicates values calculated by the direct linear plot method. Values are presented as mean±S.E.M. (*N*).

Ca^{2+}–Ringer (1 mmol l^{-1} Ca^{2+} as the divalent cation) to Mg^{2+}–Ringer's solution (1 mmol l^{-1} Mg^{2+} as the divalent cation). In both cases, the removal of either Ca^{2+} or Mg^{2+} induced a similar increase in I_{sc}. Furthermore, the stimulation of I_{sc} by removal of mucosal Ca^{2+} could be fully reversed by the addition of 1 mmol l^{-1} Ba^{2+} (Fig. 6). These experiments clearly show that the current flowing through this channel is sensitive not only to calcium but also to other divalent cations such as magnesium and barium.

To investigate the permeability of the Ca^{2+}-sensitive pathway, a series of

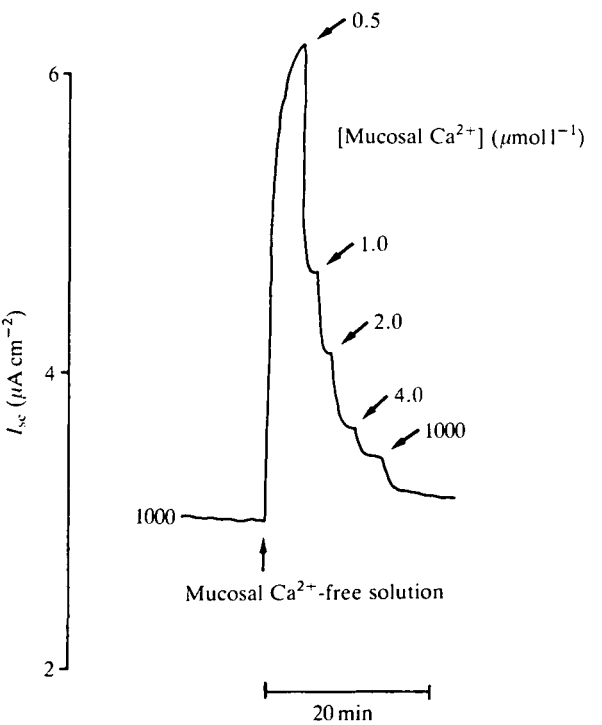

Fig. 3. Changes in the short-circuit current (I_{sc}) in response to different concentrations of mucosal Ca^{2+} at pH 8.

experiments was performed in which the main mucosal cation (usually Na^+) was replaced by other monovalent cations of the alkali metal group, such as Li^+, K^+, Rb^+ or Cs^+. Table 3 gives the values of the short-circuit current and the corresponding transepithelial conductance measured in mucosal Ringer's solutions with one of these ions as the main monovalent cation. The main cation of

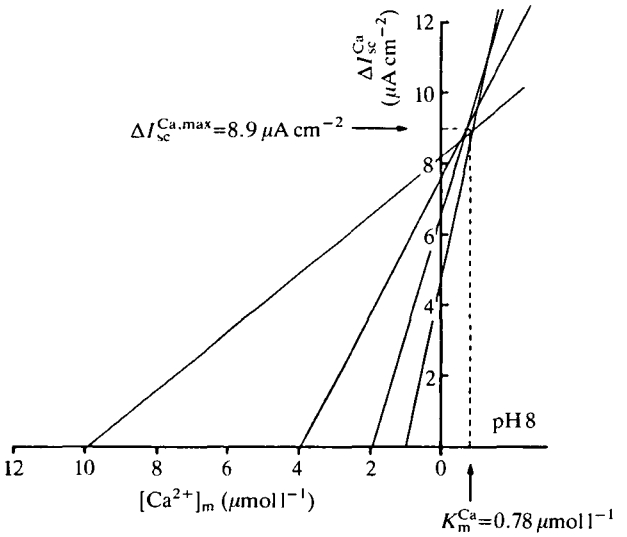

Fig. 4. An example of the calculation of the maximal Ca^{2+}-sensitive short-circuit current ($I_{sc}^{Ca,max}$) and the Michaelis constant (K_m^{Ca}) by the direct linear plot method. The data are derived from a dose–response experiment with varying mucosal Ca^{2+} concentrations ($[Ca^{2+}]_m$). For each Ca^{2+} concentration (plotted on the abscissa) the corresponding Ca^{2+}-induced inhibition of the short-circuit current (ΔI_{sc}^{Ca}) was plotted on the ordinate. Each data pair was connected by a straight line. These lines theoretically intersect (in the case of a Michaelis–Menten kinetics) at one point which gives $\Delta I_{sc}^{Ca,max}$ and K_m^{Ca}.

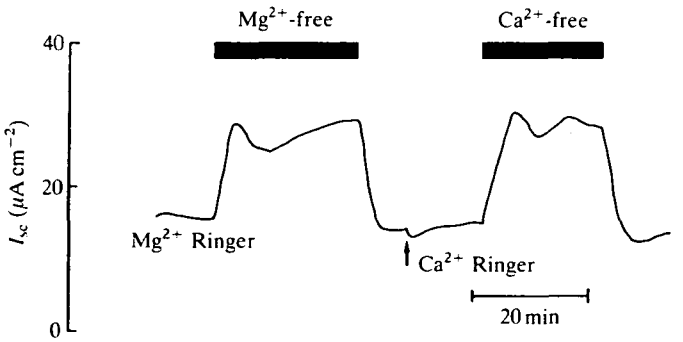

Fig. 5. Changes in the short-circuit current (I_{sc}) during the removal of the divalent cation from the mucosal solution (indicated by the bars). The concentration of the divalent cation (Mg^{2+} in Mg^{2+} Ringer, Ca^{2+} in Ca^{2+} Ringer) was $1\,mmol\,l^{-1}$. Mg^{2+} and Ca^{2+} have similar effects.

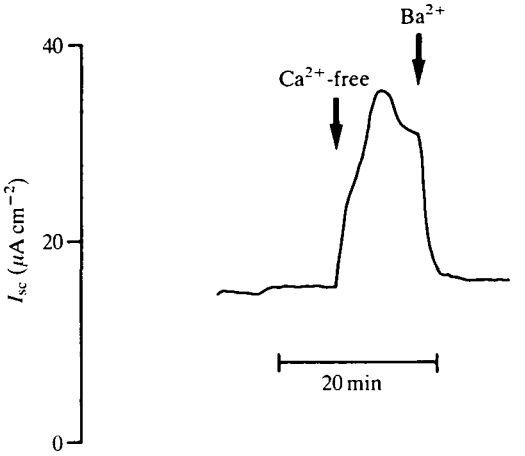

Fig. 6. Changes in the short-circuit current (I_{sc}) in an experiment in which the Ca^{2+}-sensitive increase in I_{sc} (Ca^{2+}-free mucosal solution) was abolished by the addition of 1 mmol l^{-1} Ba^{2+} to the mucosal side.

the serosal Ringer's solution was in all cases Na^+. The mucosal change from NaCl–Ringer's solution to RbCl– or LiCl–Ringer resulted in a significant decrease of short-circuit current (Table 3). In the case of CsCl–Ringer, I_{sc} also decreased in five of the six experiments, but because the results showed a wide scatter, the decrease was not found to be significant (Table 3). In contrast, the mucosal change from NaCl– to KCl–Ringer left I_{sc} relatively unchanged. It is not possible to establish the real selectivity of the ion channel for monovalent cations because the transepithelial ion movements include not only the apical but also the basolateral membrane. However, in all cases, except with lithium, a significant calcium sensitivity was found. I_{sc} increased significantly when mucosal Ca^{2+} was removed

Table 3. *Short-circuit current (I_{sc} control) and transepithelial tissue conductance (G_T control) and the increase in both parameters induced by removal of Ca^{2+} from the mucosal Ringer's solution ($\Delta I_{sc}^{Ca}, \Delta G_T^{Ca}$) when the main cation in the mucosal Ringer's solution was either Li^+, Na^+, K^+, Rb^+ or Cs^+*

	I_{sc} control	ΔI_{sc}^{Ca}	G_T control	ΔG_T^{Ca}
Li^+	10.6±2.3† (5)	1.8±0.9 (5)	0.85±0.21† (4)	0.06±0.03 (4)
Na^+	14.7±2.6 (7)	11.3±1.1* (7)	2.06±0.38 (7)	1.17±0.32* (7)
K^+	12.7±1.7 (7)	22.2±4.6* (7)	1.69±0.23 (7)	0.66±0.24* (7)
Rb^+	8.8±2.6† (7)	10.4±2.0* (7)	1.69±0.23 (7)	0.69±0.23* (7)
Cs^+	10.6±4.5 (6)	4.2±1.2* (5)	1.98±0.36 (5)	0.88±0.45 (5)

The main cation in the serosal Ringer's solution was in all cases Na^+.
Values are given as mean±S.E.M. (N).
* Significant increase over control values ($P \leq 0.05$).
† Significantly different from control values with Na^+ as the main cation ($P \leq 0.05$).

and either Na^+, K^+, Rb^+ or Cs^+ was the main mucosal cation, but the extent of stimulation varied considerably (Table 3). I_{sc}^{Ca} in the K^+–Ringer was about twice as high as in the Na^+–Ringer experiments. In contrast, when Cs^+ was the main cation, I_{sc}^{Ca} was only about 50 % of the value when Na^+ was present. Concomitant with the increase in I_{sc}, an enhanced transepithelial conductance (G_T) was measured (Table 3). After removal of mucosal Ca^{2+}, G_T was significantly higher in the Na^+-, K^+- and Rb^+-containing solutions. In the Cs^+-containing solution, the increase in G_T was not significant. In contrast, in the Li^+-containing solution, where I_{sc} was not Ca^{2+}-sensitive, G_T also remained unaffected. Obviously, this Ca^{2+}-sensitive channel is, at least in the absence of mucosal Ca^{2+}, not a specific Na^+ channel but a cation channel, permeant for several other monovalent cations, particularly K^+.

For a further pharmacological characterization of the Ca^{2+}-sensitive channel, we added several organic ion-channel blockers to the mucosal Ringer's solution. The Ca^{2+}-sensitive part of the short-circuit current was completely blocked by quinidine (Fig. 7), which is known to block basolateral K^+channels (Germann *et al.* 1986; Dawson *et al.* 1988), and by the Ca^{2+} channel blocker verapamil (Fig. 8) (Fleckenstein, 1977; Hagiwara and Byerly, 1981). The dose-dependent inhibition kinetics for both blockers seemed to be of the Michaelis–Menten type. The concentrations that induced a half-maximal inhibition were 38.4 and

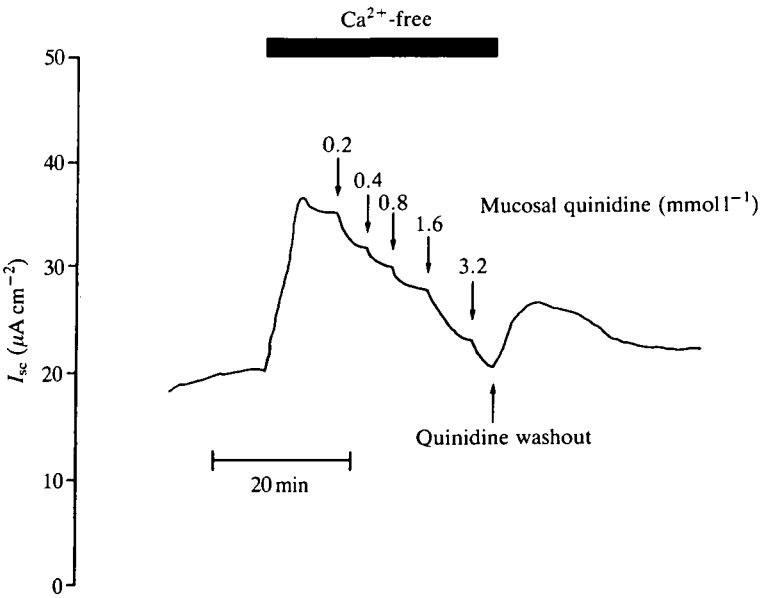

Fig. 7. Changes in the short-circuit current (I_{sc}) when it was blocked by increasing concentrations of the K^+ channel blocker quinidine added to the mucosal Ca^{2+}-free Ringer's solution. The highest quinidine concentration (3.2 mmol l^{-1}) was enough completely to reverse the increase in I_{sc} induced by removal of Ca^{2+} from the mucosal solution (indicated by the bar). The mean quinidine concentration required for a half-maximal block of the Ca^{2+}-sensitive current was 0.60±0.18 mmol l^{-1} (N=4).

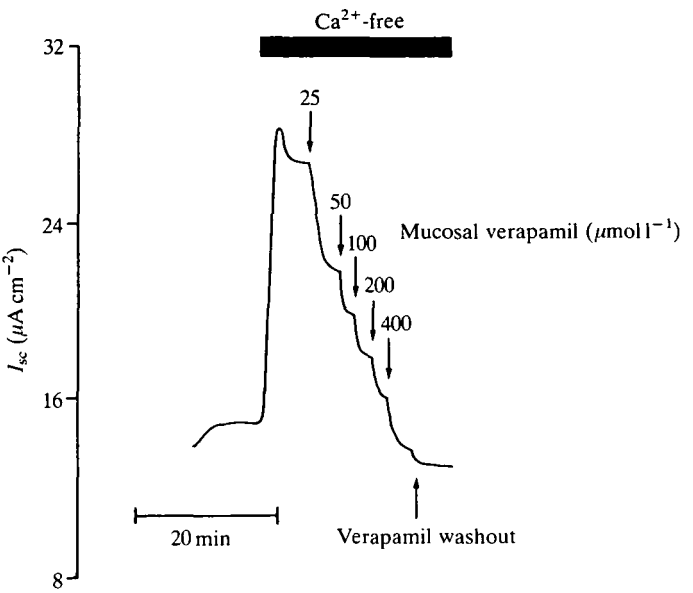

Fig. 8. Changes in the short-circuit current (I_{sc}) during the dose-dependent inhibition of the Ca^{2+}- sensitive part of I_{sc} with the Ca^{2+} channel blocker verapamil. The mucosal Ca^{2+}-free period is indicated by the bar. Half-maximal inhibition was achieved with 49.7 and 38.4 μmol l^{-1} verapamil in two experiments.

49.7 μmol l^{-1} for verapamil in two experiments, and 600±185 μmol l^{-1} (N=4) for quinidine. The effects of both quinidine and verapamil were not reversible, although reversibility would be expected for a Michaelis–Menten type of inhibition. Reversibility was only observed in one experiment when the Ca^{2+}-sensitive current was blocked by a single dose of 500 μmol l^{-1} verapamil. Surprisingly, in some experiments where the blockers were added to the serosal solution, I_{sc} decreased. However, these effects were inconsistent and small. Possibly both blockers penetrate the cell membranes and are accumulated inside the cells, which may explain the observed irreversibility and the serosal effects. Furthermore, the possibility that quinidine, and perhaps also verapamil, induces intracellular effects such as an increase in cytoplasmic Ca^{2+} concentration (Windhager and Taylor, 1983) cannot be excluded. These pharmacological data, therefore, must be interpreted with care.

Discussion

In a previous study (Krattenmacher *et al.* 1990) we demonstrated that electrogenic Na^+ absorption in the colon of the African clawed toad (*Xenopus laevis*) differs considerably from that in other vertebrates, and even that in other toads or frogs. In *Xenopus*, Na^+ passes the apical membrane of colonic epithelial cells not through the classical amiloride-sensitive Na^+ channel (Benos, 1986), but through a

channel that is sensitive to mucosal Ca^{2+}. In the present study we investigated the properties of this channel in more detail, especially with regard to its ion selectivity and its physiological role. The results of selectivity experiments of an apical ion channel have to be carefully interpreted from transepithelial measurements, because the transported ion has to pass both the apical and basolateral membranes of the epithelial cell. Furthermore, the electrochemical driving force across the apical membrane varies considerably for different ions. Thus, in accordance with common practice (Hillyard *et al.* 1982; Aelvoet *et al.* 1988), we use the term 'apparent selectivity' in this study, representing the selectivity of the epithelium for transepithelial ion-transport processes.

Our experiments clearly showed that the short-circuit current (I_{sc}) increased with the removal of mucosal Ca^{2+} when Na^+, K^+, Rb^+ or Cs^+ was the principal monovalent cation in the mucosal solution. Only when Li^+ was the main cation was there no significant Ca^{2+} sensitivity of I_{sc}. The results indicate that the junctional resistance remains unaffected by mucosal Ca^{2+} removal, provided that, in the presence of a chemical gradient, the paracellular pathway shows no cation selectivity, otherwise changes in transepithelial conductance (G_T) would also be expected in the Li^+ experiments, where no increase in Ca^{2+}-sensitive current occurred. However, although in these experiments a chemical gradient existed for Li^+ (mucosa to serosa) and Na^+ (serosa to mucosa), G_T was not increased by the change to Ca^{2+}-free mucosal solution. Thus, it seems very likely that, for the various cations, the induced current increase caused by the removal of mucosal Ca^{2+} is not due to changes in the junctional resistance but by an increased cellular conductivity. As previously shown (Na^+ as main cation), the Ca^{2+}-sensitive current increase (I_{sc}^{Ca}) is fully explained by an increase in electrogenic Na^+ transport (Krattenmacher *et al.* 1990). Therefore, it can be assumed that I_{sc}^{Ca}, in the present experiments in which Na^+ was replaced by one of the other monovalent cations, is also caused by the principal cation in the solution. With respect to the size of I_{sc}^{Ca} (Table 3), we found an apparent selectivity sequence of $K^+ > Na^+ = Rb^+ > Cs^+ > Li^+$. It is conceivable that Li^+, which is, in its hydrated form, the largest of the alkali metals (Frey-Wyssling, 1953), is too large to pass one of the cell membranes. However, from these experiments it cannot be concluded that the apical ion channel is the limiting barrier for this ion.

In the toad urinary bladder, Van Driessche *et al.* (1987) described an apical Ca^{2+}-sensitive pathway, which is permeable to Na^+, K^+, Rb^+, Cs^+, Li^+ and NH_4^+, provided that the mucosal Ringer's solution contained no Ca^{2+}. The authors found similar currents flowing through this pathway when Na^+, K^+ or Rb^+ was the principal mucosal cation, whereas Cs^+ and Li^+ induced about half as much current. The apparent ion-selectivity of this channel was $K^+ > Rb^+ = Na^+ > Cs^+ > Li^+$ (Aelvoet *et al.* 1988). We found the same sequence in the *Xenopus* colon and, therefore, a similarity between the channels seems likely. A further similarity is the strong dependence of the Ca^{2+} sensitivity on mucosal pH (Figs 1,2). For the toad urinary bladder, this increase in I_{sc}^{Ca} with decreasing proton concentration was interpreted as modulation of the channel gating (open–closed)

by protons (Aelvoet *et al.* 1988); at high pH values (low proton concentration) the channel gating may be interrupted, with the channel remaining in the open state. In the *Xenopus* colon, it is interesting to note that the inhibition kinetics of mucosal Ca^{2+} seems to be unaffected by the proton modulation of the channel gating, because the Michaelis constant of the Ca^{2+}-induced inhibition was similar at pH 6.5 and 8.0 (Table 2). Futhermore, we have shown that, in the *Xenopus* colon, this channel is not only blocked by Ca^{2+}, but also by other divalent cations such as Ba^{2+} and Mg^{2+} (Figs 5,6), providing one more similarity to the 'toad bladder' channel. A further parallel has already been described in a previous study (Krattenmacher *et al.* 1990): the Na^+ current flowing through this channel does not saturate with increasing mucosal Na^+ concentration, but depends linearly on it. All these similarities contribute to the suggestion that the Ca^{2+}-sensitive channel type present in the apical membrane of the colonic epithelium of *Xenopus* is very similar, if not identical, to the Ca^{2+}-sensitive channel type existing in the apical membrane of the epithelial cells of the toad urinary bladder. However, two differences should be mentioned: (1) whereas in the toad bladder this channel is sensitive to antidiuretic hormone (oxytocin) or its second messenger cyclic AMP (Van Driessche *et al.* 1987), these hormones have no effect in the colon of *Xenopus* (Krattenmacher *et al.* 1990); (2) in *Xenopus*, the channel has a considerable conductance even in the presence of mucosal Ca^{2+} (Krattenmacher *et al.* 1990), whereas in the toad urinary bladder the current flowing through this channel is very low when the channel is blocked by Ca^{2+}.

The physiological role of this Ca^{2+}-sensitive channel is still unclear. In recent years it has been suggested that this pathway may represent an epithelial Ca^{2+} channel (Van Driessche, 1987; Van Driessche *et al.* 1987; Aelvoet *et al.* 1988). This idea is based on the observation that the epithelial Ca^{2+}-sensitive channel has several features in common with Ca^{2+} channels found, for example, in frog muscle membranes (Almers *et al.* 1984; Almers and McCleskey, 1984; Hess *et al.* 1986): conductivity for monovalent cations in the absence of extracellular Ca^{2+}; current flow through these Ca^{2+} channels that does not saturate with increasing extracellular concentration of the permeating cation; a conductance blockable by micromolar Ca^{2+} concentrations; and sensitivity to cyclic AMP. Furthermore, Van Driessche (1987) showed that the Ca^{2+}-sensitive channel in the urinary bladder of the toad could conduct Ca^{2+} in the presence of nmol l^{-1} concentrations of Ag^+. However, the Ca^{2+}-sensitive pathway in the toad urinary bladder was recently investigated in more detail by Das and Palmer (1989), who used an appropriate method (patch-clamp) to measure the *real* ion selectivity at the single-channel level. They found a selectivity with the sequence $Rb^+=K^+>Na^+>Li^+$, which differs from the apparent selectivity described by Aelvoet *et al.* (1988). They demonstrated that the Ca^{2+} sensitivity originates from the outward rectifying characteristic of the channel: the single-channel conductance for monovalent cations was 5–6 times larger for ion movements in the cell-to-mucosa direction than in the mucosa-to-cell direction. This rectification only occurred in the presence of mucosal Ca^{2+}. In other words, the presence of extracellular Ca^{2+}

favours cation secretion over cation absorption. The authors concluded that this channel is not a Ca^{2+} channel, but the correlate of an outwardly rectifying, amiloride-insensitive apical K^+ conductance, as previously described by Palmer (1986).

For these reasons, the possibility that the channel in the *Xenopus* colon is also involved in K^+-secreting processes cannot be excluded. This hypothesis may be supported by our finding that, in the sequence of apparent ion selectivity, K^+ predominates (Table 3). Furthermore, quinidine, a blocker of basolateral K^+ channels (Germann *et al.* 1986; Dawson *et al.* 1988), completely blocked the Ca^{2+}-sensitive short-circuit current in our experiments (Fig. 7). Das and Palmer (1989) demonstrated at the single-channel level that quinidine is a potent blocker of the Ca^{2+}-sensitive pathway in toad bladder cells. This direct action of quinidine is an important finding because, in frog muscle cells, it is known that quinidine increases sarcoplasmic Ca^{2+} concentrations by reducing the Ca^{2+} uptake into intracellular stores or by inducing Ca^{2+} release from these stores (Windhager and Taylor, 1983). Although an effect of quinidine on intracellular Ca^{2+} concentration cannot be excluded, we assume that in our experiments quinidine acts directly at the Ca^{2+}-sensitive channel and not *via* changes in the intracellular Ca^{2+} concentration. This assumption is supported by the rapid action of quinidine when added to the mucosal Ringer's solution (Fig. 7). Thus, the quinidine response of the short-circuit current in the colon of *Xenopus* indicates a pharmacological similarity to either the Ca^{2+}-sensitive cation channel in the toad urinary bladder or the basolateral K^+ channel of the turtle colon. Interestingly, verapamil, which is considered to be a specific Ca^{2+} antagonist, also fully inhibited the Ca^{2+}-sensitive current (Fig. 8). However, this does not necessarily indicate that the Ca^{2+}-sensitive channel represents an epithelial Ca^{2+} channel, because the action of verapamil is not confined to Ca^{2+} channels, at least at higher ($\geq 0.1\,mmol\,l^{-1}$) concentrations (Fleckenstein, 1977; Atlas and Adler, 1981). In contrast to La^{3+}, which is known to block Na^+ transport completely (Krattenmacher *et al.* 1990), both verapamil and quinidine only blocked the Ca^{2+}-sensitive current. This may lead to reappraisal of the Ca^{2+}-sensitive pathway. The possibility that electrogenic Na^+ transport occurs through two different pathways cannot be excluded. It is conceivable that two Na^+-conducting pathways are located in the apical cell membrane: one that is not sensitive to mucosal Ca^{2+} but is blocked by La^{3+} and is always operational, and another that is totally blocked by di- or trivalent cations (Ca^{2+}, Ba^{2+}, Mg^{2+}, La^{3+}), verapamil or quinidine.

In conclusion, at present two possibilities must be considered concerning the Ca^{2+}-sensitive short-circuit current. If there is only one Na^+-conducting ion channel in the apical cell membrane it is conceivable that the effect of Ca^{2+} may be related to selectivity changes resulting from Ca^{2+} binding at or near the mouth of the channel. If there are two Na^+-conducting pathways, the Ca^{2+}-insensitive one may represent a new pathway for electrogenic Na^+ absorption in the vertebrate colon, although the physiological role of the Ca^{2+}-sensitive channel remains unclear. However, we exclude the possibility that mucosal Ca^{2+} may regulate the

channel under physiological conditions because the channel conductivity is sensitive to such extremely low extracellular Ca^{2+} concentrations. Although we have not measured the Ca^{2+} concentration of the colonic faeces, it can be assumed that, under physiological conditions, it is higher than micromolar levels.

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