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Single Chloride Channels in Colon Mucosa and Isolated Colonic Enterocytes of the Rat

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Summary. Chloride channels from rat colonic enterocytes were studied using the patch-clamp technique. After removal of mucus, inside-out patches were excised from the apical membrane of intact epithelium located at the luminal surface. They contained spontaneously switching Cl^- channels with a conductance of 35–40 pS. The channels were blocked reversibly by anthracene-9-carboxylic acid (1mM).

In excised patches from single enterocytes, isolated by calcium removal, the Cl^- channels were studied in more detail. The *I-V* relation was linear between ± 80 mV. The selectivity was $I^- > Br^- > Cl^- = NO_3^- > F^- = HCO_3^-$.

Thirty pS Cl^- channels were also found on the basolateral membrane of crypts isolated by brief calcium removal. The *I-V* curve of these Cl^- channels was also linear.

The results provide direct evidence for the existence of Cl^- channels in the apical membrane of surface cells in colonic mucosa. The properties of these channels are similar to those previously observed when incorporating membrane vesicles into planar lipid bilayers. Both results support the validity of the theoretical models describing intestinal secretion.

Key Words rat colon · Cl^- channels · anthracene-9-carboxylic acid · apical membrane

Introduction

Rat colonic mucosa responds to several neurotransmitters, bacterial toxins and mediators of inflammation with an electrogenic chloride secretion (for review *see* Binder & Sandle, 1987). The current model for this secretion includes an entrance of Cl^- into the mucosal cells from the basolateral membrane via a Na^+-K^+-$2Cl^-$ cotransporter (Frizzell & Heintze, 1979). Exit of chloride is thought to be mediated by chloride channels in the apical membrane. In general, Cl^- secretion is assumed to be localized in the crypts (Welsh et al., 1982), while the surface epithelium is thought to be the predominant site of sodium and chloride absorption. Direct evidence, however, for Cl^- channels in the apical membrane of native enterocytes and their distribution between crypt and surface cells is not available.

Recently a 40–50 pS anion channel was found when vesicles from homogenized colonic enterocytes were fused with planar lipid bilayers (Reinhardt et al., 1987). Unavoidably, a mixture of apical and basolateral membrane vesicles was used in this study. The patch-clamp technique (Hamill et al., 1981), however, allowed us to search for the localization of these Cl^- channels and to study their properties in the natural lipid environment.

Materials and Methods

TISSUE PREPARATION

Wistar rats of both sexes weighing 200–250 g were used. The animals had free access to food and water until the day of the experiment. Animals were killed by cervical dislocation.

Muscularis propria and submucosa of the colon descendens were removed as described by Andres et al. (1985). The mucosa was fixed on a plastic holder with tissue adhesive (histoacryl blue, Braun-Melsungen, Melsungen, FRG) and transferred into a Tyrode solution containing (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.25; $MgCl_2$, 1; HEPES, 10; and adjusted by NaOH to a pH of 7.4.

The holder was transferred into the experimental chamber, which was perfused hydrostatically throughout the experiment. For patch clamping, the chamber was placed on the stage of an inverted microscope (Olympus IMT2-F). After gently blotting the mucosa with filter paper and washing with the sulfhydryl agent dithiothreitol (3 mM) for 10 min to remove mucus, patch pipettes could be sealed onto the apical membrane of the surface epithelium.

CELL ISOLATION

In a second set of experiments, enterocytes were isolated using a low calcium protocol based on the method described by Bridges et al. (1986) and Reinhardt et al. (1987) and modified for the patch-clamp experiments. The mucosa was prepared as described above, fixed on a plastic holder and transferred into a

Table. Composition of modified Tyrode solutions (mM)

Symbol	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	J^-	Br^-	F^-	NO_3^-	HCO_3^-	Gluconate	SO_4^{2-}	HEPES	Mannitol
NaCl	140	5.4	1.25	1	149.9	—	—	—	—	—	—	—	10	—
½ NaCl	70	—	1.25	2	76.5	—	—	—	—	—	—	—	10	140
NaJ	140	5.4	1.25	1	9.9	140	—	—	—	—	—	—	10	—
NaBr	140	5.4	1.25	1	9.9	—	140	—	—	—	—	—	10	—
NaF	140	5.4	1.25	1	9.9	—	—	140	—	—	—	—	10	—
$NaNO_3$	140	5.4	1.25	1	9.9	—	—	—	140	—	—	—	10	—
$NaHCO_3$	140	5.4	1.25	1	9.9	—	—	—	—	140	—	—	10	—
Na gluc	140	5.4	1.25	1	9.9	—	—	—	—	—	140	—	10	—
Na_2SO_4	140	5.4	1.25	1	9.9	—	—	—	—	—	—	70	10	—
KCl	—	140	0.3	2	144.6	—	—	—	—	—	—	—	10	—

Ca^{2+}-free Parsons solution containing (in mM): NaCl, 107; KCl, 4.5; NaH_2PO_4, 0.2; Na_2HPO_4, 1.8; $NaHCO_3$, 25; EDTA, 5; glucose, 12. The solution was gassed with 5% CO_2 in 95% O_2 and was buffered with Tris to a pH of 7.4. The mucosa was kept at 37°C during the isolation procedure.

Every 5 min, the epithelial cells were removed from the tissue using a 15-sec vibration (Vibromixer El, Chemap AG, Switzerland) and collected in pregassed Parsons buffer of room temperature. All further steps including the patch experiments were carried out at room temperature.

Cells were washed twice by centrifugation (3 min, 500× *g*) and transferred into a Tyrode solution (*see above*) containing 12 mM glucose and 5 mM sodium pyruvate as substrate. The enterocytes were pipetted into the experimental chamber, a silicon ring attached to a glass slide. The volume of the chamber was about 0.1 ml. All solutions for the isolation or storage of the cells except those used for patching contained 0.1% bovine serum albumin, which is known to have a "protective" effect on isolated enterocytes (Perris, 1965).

During isolation, most of the enterocytes rounded off and attached to the glass bottom of the chamber. Thirty min after isolation of the cells the enterocytes were washed for 10 min with 3 mM dithiothreitol at pH 7.4. The cells were superfused hydrostatically throughout the experiment. Perfusion rate for isolated cells was about 1 ml · min^{-1}, i.e., one chamber volume every 6 sec.

ISOLATION OF CRYPTS

Crypts were prepared by a modification of the cell isolation procedure similar to the method described by Bjerknes and Cheng (1981). The mucosa was fixed on a holder with histoacryl, transferred for 15 min in a Ca^{2+}-free Parsons solution (*see above*) containing 0.1% bovine serum albumin and vibrated once for 30 sec. This led to the isolation of intact crypts. They could be fixed on the glass of the experimental chamber with the aid of poly-L-lysine (5 mg · ml^{-1}). After washing with 3 mM dithiothreitol, gigaohm seals could be obtained on the basolateral membrane.

ELECTRICAL RECORDING

Patch-clamp experiments were performed following Hamill et al. (1981). Patch pipettes were pulled from thick-walled borosilicate glass capillaries (Jencons Scientific Ltd., Bedfordshire, UK, outer diameter 2 mm, inner diameter 1 to 1.25 mm) on a two-stage puller designed in the laboratory. After fire-polishing, the tips had resistances of 5 to 10 MΩ when filled with Tyrode.

The pipettes were manipulated with a hydraulic 3-D manipulator (Narishige MO-103N, Tokyo, Japan). During approach to the cells, the pipette interior was held under positive pressure. Patch-clamp currents were recorded with an amplifier of our own design. Current and voltage signals were digitized at 40 kHz (16 bits) with an audio-PCM processor (Sony 501 ES, modified to pass zero frequency) and stored on a video recorder. Patch potentials were referred to the extracellular side of the membrane.

Sealing of the pipette on the cell surface was aided by gentle suction. Seal resistances were 2 to 8 GΩ. Sealing could be improved by superfusing the cells with a Tyrode containing 10 mM $CaCl_2$. This solution was washed away once the seal had formed.

Patch pipettes were usually filled with NaCl-Tyrode while the cells were superfused with a KCl-Tyrode (Table). For experiments with intact mucosa and for ion substitution experiments, pipettes were filled with KCl-Tyrode and the cells were perfused with the sodium salts of the corresponding anions (Table). The Ca^{2+} concentration at the cytosolic site of the patch was kept relatively high during the experiments in order to improve the seals.

MORPHOLOGY

For morphological examinations, crypts and isolated cells were prepared as described above except that they were collected in a Tyrode buffer containing 12 mM glucose, 5 mM sodium pyruvate, 3 mM dithiothreitol and 0.1% bovine serum albumin. Sediments containing both crypts and isolated epithelial cells were prepared for scanning electron microscopy following two schedules. In the first one, the sediment was fixed in suspension in a solution containing 3% glutaraldehyde and 1% formaldehyde buffered with 0.06 M phosphate at pH 7.35 during 2 hr. After an additional fixation with 1% osmium tetraoxide, the sediment was embedded in agar (2%). Agar blocks containing tissue sediment were post-fixed again in 1% glutaraldehyde overnight at 8°C. Microchopper sections, 60–100 μm thick, were dehydrated and critical point dried using CO_2.

In the second procedure the sediment was fixed 25 min at room temperature in a 1 : 1 suspension of Tyrode and the fixative described above containing glutaraldehyde and formaldehyde. After washing in phosphate buffered saline, the sediment was spread on poly-L-lysine coated slides and kept for 30 min in a humide chamber. The binding of the cells to the poly-L-lysine was stabilized by an additional treatment with glutaraldehyde

(2.5%) in distilled water for 40 min at room temperature. After fixation with osmium tetraoxide (0.5%), the slides were dehydrated and critical point dryed. Agar sections and slides were coated with gold (2–5 nm thick) and were examinated in a scanning electron microscope (Cam Scan E2).

CHEMICALS AND STATISTICS

Values are given as means ± 1 SEM. Significances of differences were tested by unpaired, two-tailed Student's *t* test. Anthracene-9-carboxylic acid (A-9-C, Aldrich) was dissolved in DMSO (final DMSO concentration 0.2%), which itself had no effect on channel activity. Poly-L-lysine (mol wt > 300,000) was obtained from Sigma, St. Louis, MO.

Results

APICAL SURFACE OF INTACT MUCOSA

Single-channel records from the surface of intact intestinal epithelium are difficult to obtain because sealing of the pipette to the membrane requires a clean membrane surface. Therefore, the mucus had to be removed from the preparation. This was attempted by gentle blotting of the luminal surface with filter paper and subsequent rinsing with 3 mM dithiothreitol in Tyrode to solubilize the mucus. The effectiveness of the removal of mucus by dithiothreitol can be recognized in Fig. 1*a*, where the apical surface of the epithelium is depicted after treatment with a washing protocol similar to the protocol used for the patch-clamp experiments. In addition, the rate of perfusion through the experimental chamber was increased to about 3 ml · min^{-1} to remove mucus freshly produced in the crypts during the experiment.

Following these steps, patch pipettes could be sealed on the apical membrane of surface cells in intact preparations in 11 out of 160 trials. In these 11 patches, 20 channels were observed. Three of them had a reversal potential between 80 and 60 mV (K$^+$ reversal potential 86 mV for these conditions, i.e., 140 mM KCl in the pipette and 140 mM NaCl with 5.4 mM KCl in the perfusion medium) and slope conductances of 120–150 pS, indicating K$^+$-selective channels (data *not shown*). The other 17 channels were of the type shown in Fig. 5*A*. They were active during cell-attached recording as well as after excision of the inside-out patch. The reversal potential was near 0 mV and was thus close to the chloride reversal potential (+0.95 mV) under these conditions. Their *I-V* relation showed a pronounced rectification on hyperpolarization of the patch (Fig. 5*B*). Linear fit of the data between −30 and +50 mV gave a single-channel conductance of 39.8 ± 5.0 pS, whereas a linear fit in the range of −80 to −30 mV revealed a slope conductance of only 13.5 ± 1.7 pS.

These channels could be blocked reversibly by the chloride channel blocker anthracene-9-carboxylic acid (A-9-C). Figure 6 shows the decrease in current of an inside-out patch after application of A-9-C (first arrow). Total patch current decreased by 18 pA with a single-channel current amplitude of about 2 pA. On removal of A-9-C from the bathing solution (Fig. 6, second arrow), channel activity recovered partially. The efficacy of blockage with A-9-C is known to be low. Thus, the high concentration of 1 mM was required to achieve a complete block. With regard to the blockability by A-9-C and the reversal potential, these channels behave as theoretically expected for Cl$^-$ channels.

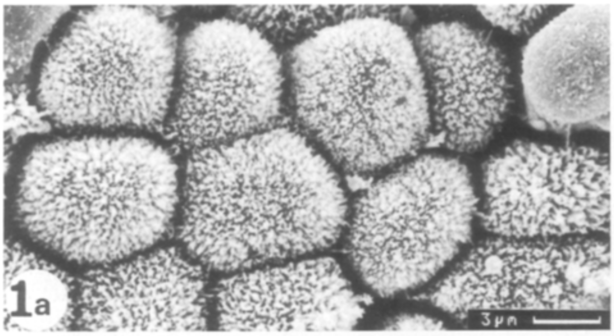
ISOLATED CELLS

Because of the low sealing rate (7%) with intact mucosa, we used isolated enterocytes for further characterization of the presumed Cl$^-$ channel. With single cells, gigaohm seals were obtained in about 90% of the trials. A distinction between apical and basolateral patch-localization was not possible, because the isolated enterocytes rounded off (Figs. 3, 4). It was also not possible to distinguish between surface and crypt cells.

The channel type found dominantly ($n = 47$) with this method was an anion channel with a linear *I-V* relation between +80 and −80 mV (Fig. 7). The reversal potential was 0 mV. The single-channel conductance scattered from 25 to 50 pS with a mean value of 34 ± 0.5 pS (measured at the reversal potential). Thus, the *I-V* relation differs from the channel found in the intact mucosa, but the conductances at the reversal potential are nearly identical. Conductance substates were not observed.

The linear *I-V* relation and the reversal of 0 mV in NaCl: KCl (and vice versa, *see* Fig. 9) indicates that the channel is either a Cl$^-$ channel, or a cation channel, which does not discriminate between sodium and potassium. Therefore, the anion to cation selectivity was determined. Changing from a NaCl-Tyrode on the cytosolic side of the patch to a NaCl-Tyrode with only half of the concentration of NaCl ($\frac{1}{2}$ NaCl, *see* the Table) shifted the reversal potential from 0 mV to −17.6 ± 1.2 mV ($n = 7$, *see* Fig. 8). From the equation

$$E_{\mathrm{rev}} = -61 \cdot \log \frac{[\mathrm{anion}]_o \cdot P_{\mathrm{anion}} + [\mathrm{cation}]_i \cdot P_{\mathrm{cation}}}{[\mathrm{anion}]_i \cdot P_{\mathrm{anion}} + [\mathrm{cation}]_o \cdot P_{\mathrm{cation}}} \quad (1)$$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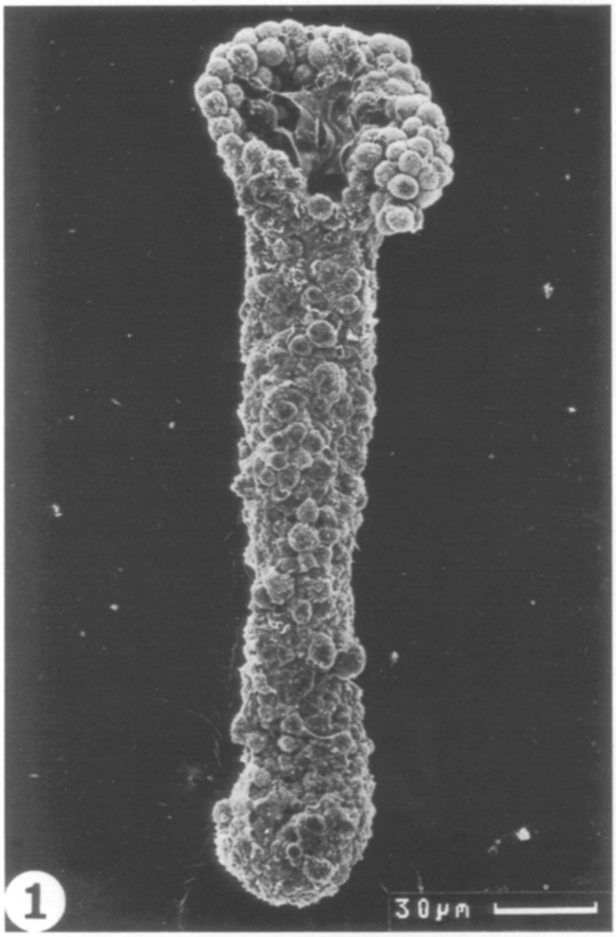
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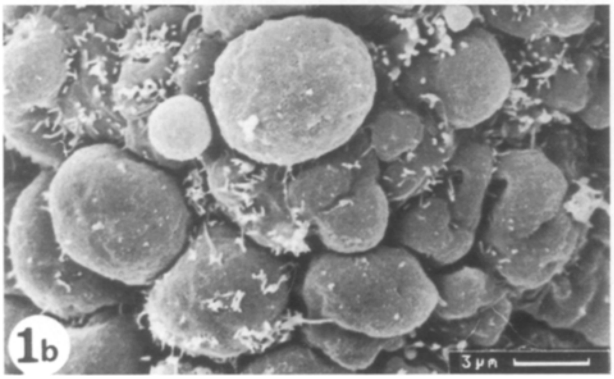
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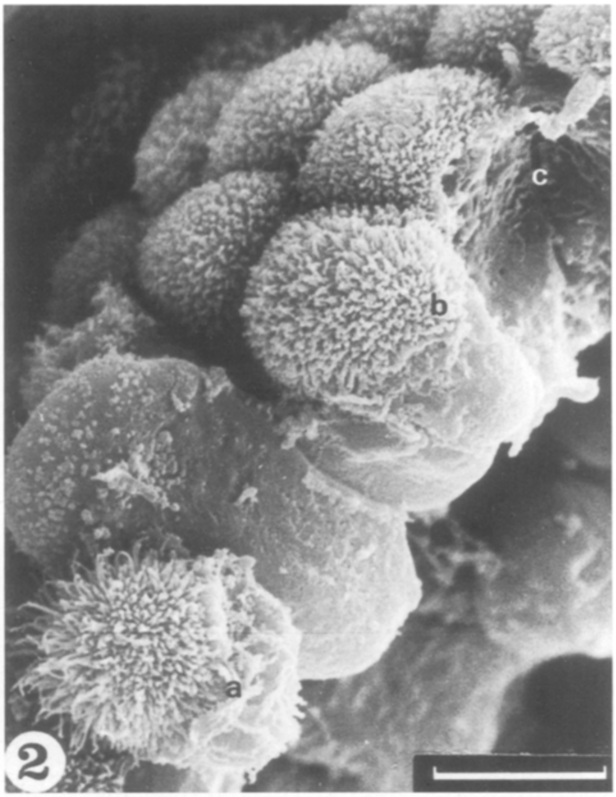
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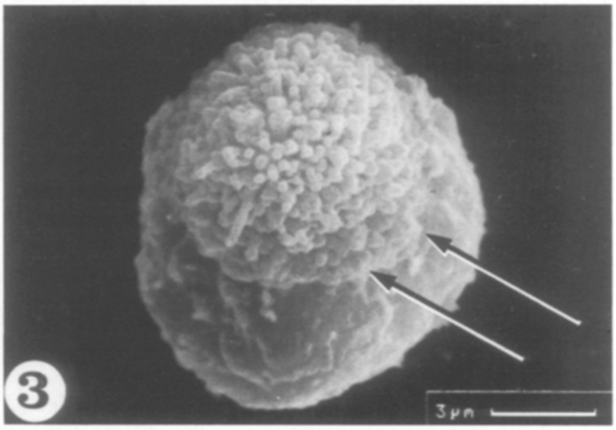
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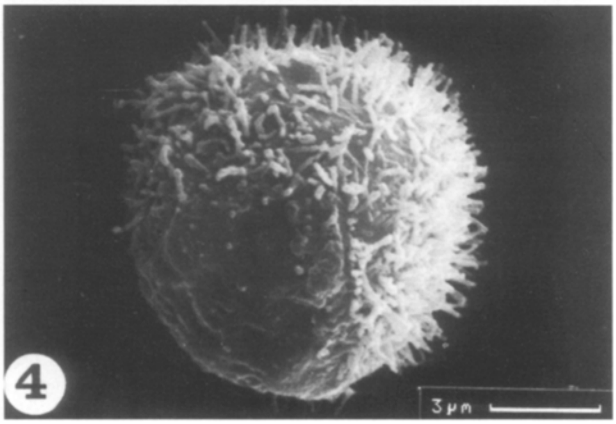
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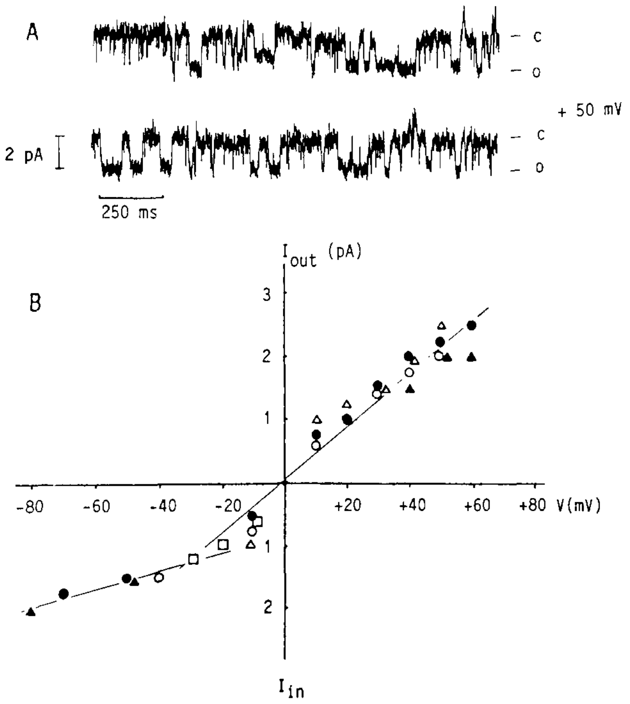

Fig. 5. (*A*) Spontaneous switching of a 35-pS anion channel. Inside-out patch excised from the apical membrane of the surface epithelium (*c* = closed state, *o* = open state). Plotting bandwidth was 1 kHz. (*B*) *I-V* relation of five 35-pS Cl$^-$ channels from the same location, an inside-out patch. Data from different individual channels were represented by different symbols. Slope of the regression lines: *a* = 39.8 ± 5.0 pS; *b* = 13.5 ± 1.7 pS

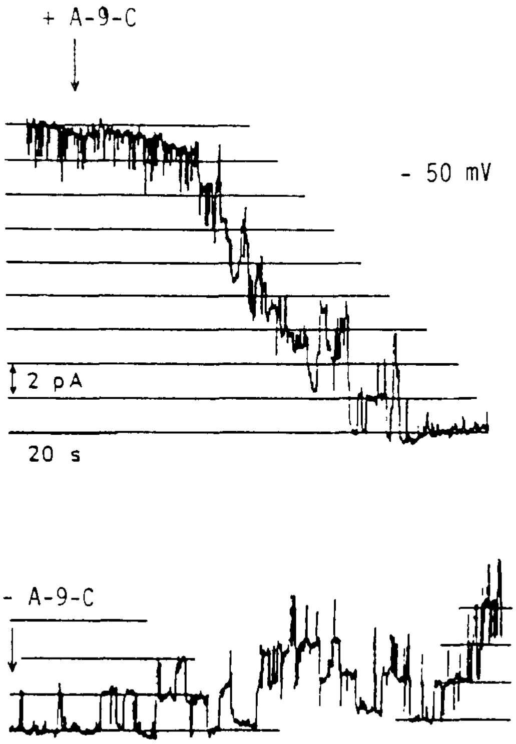

Fig. 6. Block of nine 35-pS Cl$^-$ channels in an inside-out patch obtained from intact mucosa by a fully blocking concentration of A-9-C. 1 mM A-9-C was added at the first arrow and removed at the second arrow. The current signal is "contaminated" by a drift, but allows recognition of several equal steps of current decrease following addition of A-9-C. Plotting bandwidth was 2 kHz

a cation to anion permeability of 0.01 was calculated. From Fig. 8 it can also be recognized that replacement of Cl$^-$ on the cytosolic side of the patch with gluconate abolishes the inward current (carried by Cl$^-$ moving from the bath into the pipette) indicating that the channel is impermeable for this large anion.

The anion selectivity of the channel was determined in ion substitution experiments, replacing NaCl on the cytosolic side of the patch by the sodium salts of the anions to be tested (*see* the Table for composition of the solutions). The Cl$^-$ concentration in the pipette was kept constant. From the shift of the reversal potential, the selectivity for different anions was calculated (Fig. 9). The selectivity of the channel (relative permeabilities with $P_{Cl} = 1$) was

$$I^- \ (1.4) > Br^- \geq Cl^- = NO_3^- \ (1) > F^- = HCO_3^- \ (0.5)$$

Fig. 1. Crypt from rat colon descendens isolated by brief (15 min) Ca^{2+} removal. The opening of the crypt is oriented upward, mucus remaining in the crypt is visible. (*a*) Apical surface of the epithelial cells at higher magnification. Note removal of mucus allowing a clear image of the brush border. (*b*) Basal surface of the epithelial cells. Visible are relatively bare basal cell poles and the intercellular gaps with processes possibly derived from cellular interdigitations. The basal lamina is almost completely removed

Fig. 2. Apical and lateral surface of epithelial cells (agar-section) near the opening of a crypt. Of the cells (*a*, *b*, *c*) at the edge, *b* and *c* still have their columnar shape and *c* shows a concave surface, which may be an impression from an adjacent cell. On the left, a cell with a low density of microvilli at the apical surface can be recognized, which could be a resting goblet cell. Bar indicates 6 μm

Fig. 3. Single cell isolated by long (30 min) Ca^{2+} removal. Apical cell pole still clearly deliminated (arrow)

Fig. 4. Isolated cell where more than half the cellular surface is covered by apical-type microvilli

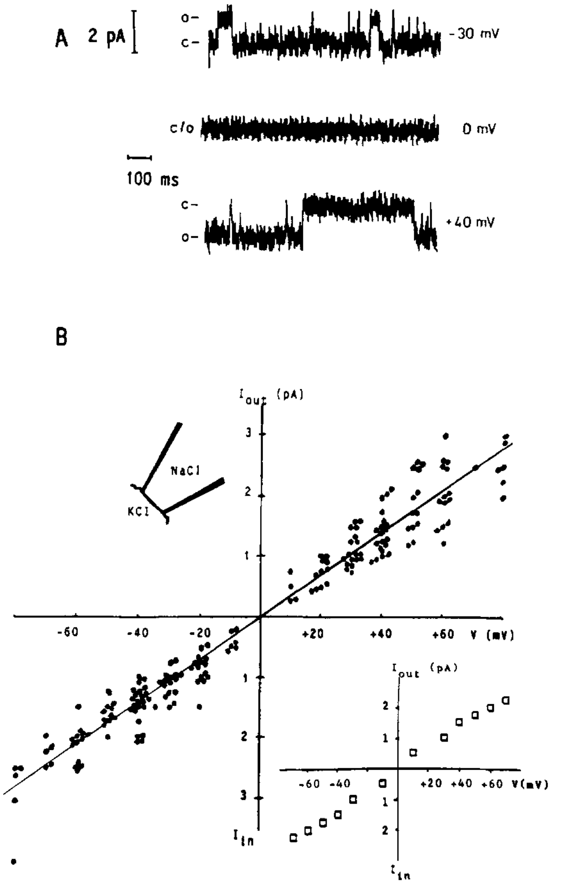

Fig. 7. (*A*) Spontaneous switching of a 35-pS anion channel. Inside-out patch was from isolated enterocytes (*c* = closed state, *o* = open state). Plotting bandwidth was 2 kHz. (*B*) *I-V* relation of 24 Cl⁻ channels from isolated cells obtained from records like those of *A*. The slope of the regression line indicates a mean channel conductance of 34 ± 0.5 pS. In the inlet, the *I-V* curve of one of these Cl⁻ channels is depicted for better demonstration of the linear *I-V* relation

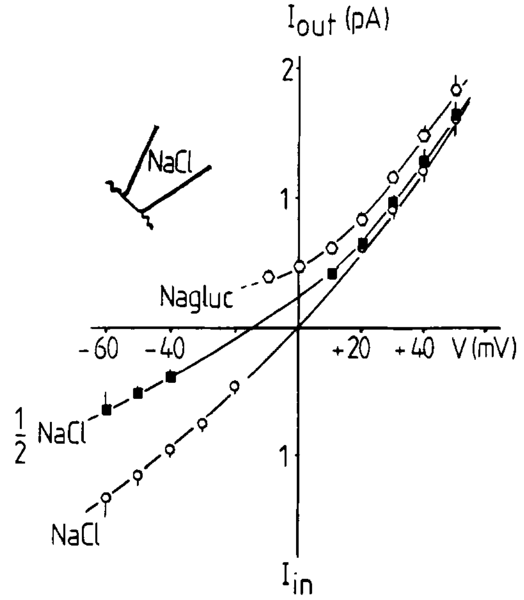

Fig. 8. *I-V* relation of 35-pS Cl⁻ channels in NaCl-Tyrode, in a Tyrode, where the NaCl concentration is reduced to about 50% (½ NaCl, osmolarity compensated by the addition of mannitol), and in Na gluconate-Tyrode (composition of solutions *see* the Table). In the Na gluconate-Tyrode no single-channel events could be resolved at holding potentials more negative than −10 mV. Inside-out patches were from isolated enterocytes. Lines were drawn by hand to interpolate the reversal potentials. Values are means ± SEM, *n* = 6–7 for each condition

calculated from

$$E_{rev} = -61 \cdot \log \frac{[Cl^-]_o \cdot P_{Cl} + [X^-]_o \cdot P_X}{[Cl^-]_i \cdot P_{Cl} + [X^-]_i \cdot P_X} \qquad (2)$$

where X^- is any anion.

The pH of the $NaHCO_3$-Tyrode (Table) was 7.8. A shift in pH from 7.4 to 7.8 had no effect on the *I-V* relation of the Cl⁻ channel in control experiments. Reversal was not reached when gluconate or sulfate substituted for chloride, indicating that these large anions will not pass the channel (*see also* Fig. 8).

The 35-pS Cl⁻ channel was blocked with A-9-C applied to the cytosolic side of the patch (Fig. 10). This was tested in 9 patches containing a total number of 19 Cl⁻ channels, where a concentration of 4 mM in initial experiments, and in later experiments 1 mM A-9-C, always caused a complete and reversible block of the channel activity. During washout, short channel openings were observed with no detectable changes in single-channel current amplitude[1] (Fig. 10).

Infrequently (five cases), an anion channel of 344 ± 16 pS conductance was observed (Fig. 11). This channel was also selective for Cl⁻ over gluconate (*n* = 1).

Anion channels of similarly large conductance have been found in rat muscle (Blatz & Magleby, 1983) and cultured kidney cells (Nelson, Tang & Palmer, 1984). As only a few records in our material contained this channel, we cannot exclude that this

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[1] This is in contrast to the mode of action of A-9-C in tracheal epithelium, where A-9-C was found to reduce single-channel currents in a dose-dependent fashion (Welsh, 1986). The latter observation could be explained by A-9-C acting as a high-rate blocker in tracheal cells. Because of the limited bandwidth of recording, rapid flickering of the true channel current will cause reduction of the recorded single-channel current.

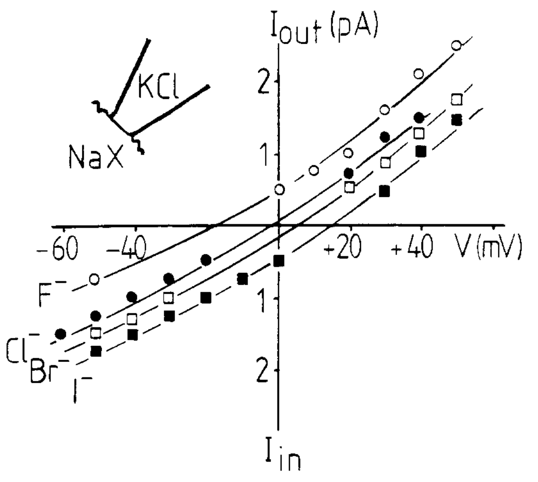

Fig. 9. *I-V* relation of a 35-pS Cl^- channel during chloride replacement with other halogens. Inside-out patch was from isolated enterocytes. Lines were drawn by hand to interpolate the reversal potentials. Representative for 4–7 *I-V* curves obtained for each halogen. The mean fitted reversal potential for Cl^- on both sides was -3.5 ± 1.2 mV, which may represent an offset potential. Replacement with F^-, Br^- and J^- shifted the reversal potential by -15.5 ± 2.7 mV ($P < 0.01$), $+1.6 \pm 1.9$ mV (not significant) and $+9.9 \pm 2.3$ mV ($P < 0.01$), respectively (deltas to the fitted Cl^- reversal potential)

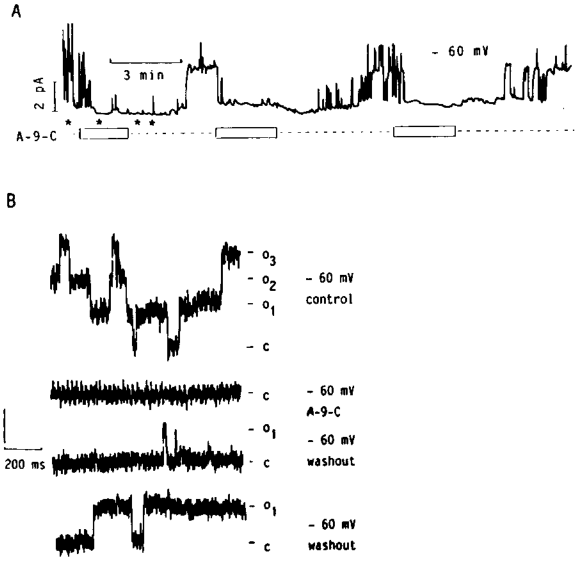

Fig. 10. (*A*) Block of 35-pS Cl^- channels from isolated cells by 4 mM A-9-C. Inside-out patch was from isolated enterocytes containing at least 3 channels (*c* = closed state, *o* = open state). (*B*) Current trace during first A-9-C application (indicated by *) from the same patch plotted with a different time scale. Plotting bandwidth was 2 kHz

channel has conductive substates as reported for other large anion channels, even though we did not observe them.

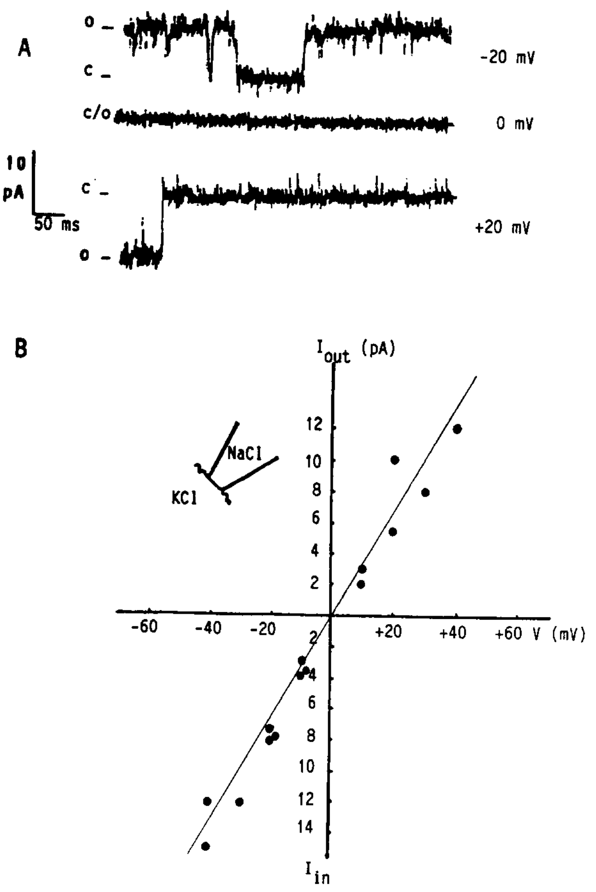

Fig. 11. (*A*) Spontaneous open/close events of one Cl^- channel of large conductance from isolated enterocytes. Inside-out patch was from isolated enterocytes (*c* = closed state, *o* = open state). Plotting bandwidth was 10 kHz. (*B*) *I-V* relation of five large Cl^- channels. The regression line indicates a mean single-channel conductance of 340 ± 16 pS

BASOLATERAL SURFACE OF ISOLATED CRYPTS

The above results raised the question, whether the Cl^- channels found in the isolated cells were of apical origin, or whether also in the basolateral membrane Cl^- channels exist, which have a linear *I-V* relation. Several attempts to isolate intact crypts were made. Trials with collagenase or mechanical removal of the lamina propria were unsuccessful, because removal of connective tissue was incomplete. However, by brief (15 min) exposure to calcium-free solution, isolated crypts could be prepared. Their cells exposed a "clean" basolateral membrane free of basal lamina and allowed a clear differentiation between apical and basal cell pole (Fig. 1). In all crypts where the basal lamina was successfully removed, a slight protrusion of the basolateral cell pole was recognized in the electron microscope. This protrusion did not occur in crypts with an adherent basal lamina (*not shown*), indicating a supporting function of the basal lamina for cell shape. After some time (between 1–4 hr for different crypts) cell rounding was also observed in these crypts. It was more pronounced near the opening of the crypt (Fig. 2), while in many cases rounding was not seen in the middle segments of the crypt even after several hours. Patch experiments were carried out before changes in cell shape were visible. From 28 patches obtained from the basolateral membrane of intact crypts, 5 contained Cl$^-$ channels. The *I-V* relation of these channels was linear with a slope conductance of 29 ± 0.8 pS (Fig. 12). They could also be blocked with 1 mM A-9-C ($n = 3$) and were not permeable for gluconate ($n = 2$, *data not shown*).

Discussion

Cl$^-$ CHANNEL PROPERTIES

The single-channel conductance and the ionic selectivity of the 35-pS chloride channel found in isolated enterocytes, from which most of our data were obtained, closely resemble the properties of the chloride channel incorporated into planar phospholipid bilayers (Reinhardt et al., 1987). In both cases the permeability for different halogen ions is inversely proportional to the ionic size (crystal radius), indicating that a partial loss of the hydration shell of the ion during passage through the channel is rate limiting[2]. The channels differ, however, in the voltage-dependence of their open probability. In the planar bilayer the channel "turned off" at voltages exceeding +50 or −50 mV, while in its natural lipid environment (the membrane patch) the channel switched spontaneously even at high voltages and had a linear *I-V* curve between ±80 mV.

The conductance of this channel is not much different from that of Cl$^-$ channels observed in dogfish rectal gland (Greger, Schlatter & Gögelein, 1985; 1987), human trachea (Frizzell, Rechkemmer & Shoemaker, 1986; Welsh, 1986; Welsh & Liedtke, 1986), canine trachea (Shoemaker et al., 1986) and in cultured human colonic tumor cells (Hayslett et al., 1987; Halm et al., 1988), where anion channels of 25–50 pS have been described. Thus anion channels of similar conductance seem to be a common feature of many secretory epithelia.

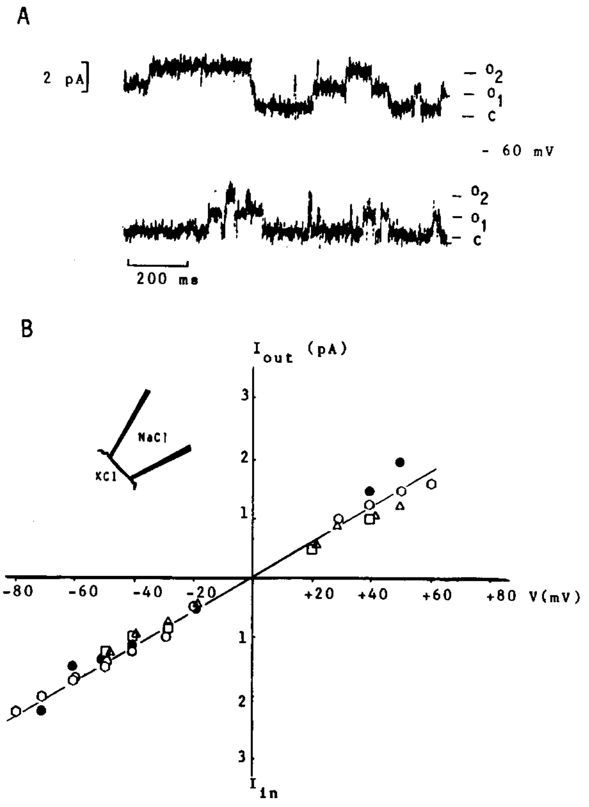

Fig. 12. (*A*) Spontaneous open/close events of two Cl$^-$ channels from the basolateral membrane of intact crypts. Inside-out patch (c = closed state, o = open state). Plotting bandwidth was 1 kHz. (*B*) *I-V* relation of five Cl$^-$ channels from the basolateral membrane. Data from different individual channels represented by different symbols. The regression line indicates a mean single-channel conductance of 29 ± 0.8 pS

MORPHOLOGICAL AND FUNCTIONAL RESPONSE TO CELL ISOLATION

An interesting phenomenon is the spreading of membrane material on the surface of isolated enterocytes. As studied in detail by Ziomek, Schulman and Edidin (1980) and Bjorkman et al. (1986), within a few minutes after removal of the enterocytes from the mucosa, marker proteins for brush-border membrane distribute uniformly over the whole cellular surface. Thus the asymmetry between the brush-border and the basolateral membrane is lost following disruption of the zonula occludens. A spreading of apical membrane components after isolation of the enterocytes was also found in our material, as indicated by a distribution of microvilli over more than half of the cellular surface in some cells (Fig. 4). This may explain why we observed Cl$^-$ channels in most patches from the surface of the single cells. Apical and baso-

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[2] In contrast, the Cl$^-$ channel of the human colonic tumor cell HT 29 has a larger conductance (50 pS) and does not discriminate between halogen ions (Hayslett et al., 1987).

lateral membranes differ in their lipid composition (Brasitus & Keresztes, 1984). The disappearance of this particular distribution of membrane lipids after cell isolation may be the reason for the loss of the rectification of the *I-V* curve on hyperpolarization observed with patches excised from the isolated enterocytes compared to patches derived from the apical surface of intact epithelium (*cf.* Figs. 5 and 8). Effects of the lipid phase on the properties of transport proteins like the glucose-Na$^+$ cotransporter (Fernandez et al., 1984) or the Na$^+$/H$^+$ exchanger (Brasitus et al., 1986) are well known.

DISTRIBUTION AND POSSIBLE FUNCTION OF Cl$^-$ CHANNELS

Another possibility is that the basolateral membrane normally contains Cl$^-$ channels, and that these have an *I-V* relation somewhat different from that of apical Cl$^-$ channels. Therefore, patch-clamp experiments were carried out at the basolateral membrane of crypts isolated by brief Ca^{2+} removal. The cells in several crypts rounded after some time. While the experiments were performed only at those parts of the crypts where no rounding was visible, the possibility remains that even here channels had spread from the apical membrane, i.e., that partial opening of the tight junction (due to the Ca^{2+} removal) had allowed spreading before changes in cell morphology became noticeable in the light microscope. The *I-V* relation of these channels differed somewhat from that of apical Cl$^-$ channels in that it was linear. Possibly these channels were *a priori* localized in the basolateral membrane. However, we cannot exclude that they had spread from the apical membrane, changing their *I-V* curve in the changed lipid environment.

One is tempted to speculate about a possible function of a basolateral Cl$^-$ channel, which could be an involvement in volume regulation or in electrogenic Cl$^-$ exit through the basolateral membrane during Cl$^-$ absorption. NaCl is absorbed electroneutrally by the colon and this is mediated in the apical membrane by a Na$^+$/H$^+$ and a Cl$^-$/HCO$_3^-$ exchanger working in parallel (Binder & Sandle, 1987). As Na$^+$ is thought to leave the cell by the electrogenic 3Na$^+$/2K$^+$ pump and K$^+$ recycles by basolateral K$^+$ channels, Cl$^-$ movement through the basolateral membrane should be electrogenic to keep the transcellular absorption of NaCl electroneutral. The existence of a basolateral Cl$^-$ conductance was already postulated in the model for NaCl absorption in rat proximal colon by Lübcke et al. (1986).

In intact mucosa, the Cl$^-$ channels were found on the apical membrane of surface cells. Our data do not allow us to answer the question, whether these channels also exist on cells within the crypts, where apical patching was not possible. However, the crypts are well known for their strong chloride secretion (Welsh et al., 1982). Since the surface cells derive from the crypt cells (for review *see* Lipkin, 1981), it is not surprising that the A-9-C-blockable Cl$^-$ conductance is not restricted to the crypt cells. This was previously indicated by the microelectrode studies of Horvath et al. (1986) in rabbit colon. A similar situation in the small intestine was described recently by Giraldez, Sepúlveda and Sheppard (1988), who observed electrophysiological correlates of secretion and absorption in the same cell, suggesting that the separation between absorbing and secreting parts of the intestinal epithelium may not be as strong as previously assumed.

In conclusion, evidence is presented for the existence of Cl$^-$ channels in the apical membrane of colonic enterocytes. While this channel location was postulated in several previous models for intestinal secretion (Frizzell & Heintze, 1979; Binder & Sandle, 1987), it was generally specified for crypt cells (Welsh et al., 1982; Horvath et al., 1986). Now it appears that the Cl$^-$ channels are not restricted to the crypt cells but exist also in the surface epithelium. Thus the surface cells may well participate in colonic secretion. The origin of the observed basolateral Cl$^-$ conductance still needs further investigations.

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References

Andres, H., Bock, R., Bridges, R.J., Rummel, W., Schreiner, J. 1985. Submucosal plexus and electrolyte transport across rat colonic mucosa. *J. Physiol.* (*London*) **364:**301–312

Binder, H.J., Sandle, G.I. 1987. Electrolyte absorption and secretion in the mammalian colon. *In*: Physiology of the Gastrointestinal Tract. L.R. Johnson, editor. pp. 1389–1418. Raven, New York

Bjerknes, M., Cheng, H. 1981. Methods for the isolation of intact epithelium from the mouse intestine. *Anat. Rec.* **199:**565–574

Bjorkman, D.J., Allan, C.H., Hagen, S.J., Trier, J. 1986. Structural features of absorptive cell and microvillus preparations from rat small intestine. *Gastroenterology* **91:**1401–1414

Blatz, A.L., Magleby, K.L. 1983. Single voltage-dependent chloride-selective channels of large conductance in cultured rat muscle. *Biophys. J.* **43:**237–241

Brasitus, T.A., Dudeja, P.K., Worman, H.J., Foster, E.S. 1986. The lipid fluidity of rat colonic brush-border membrane vesicles modulates Na^+-H^+ exchange and osmotic water permeability. *Biochim. Biophys. Acta* **855:**16–24

Brasitus, T.A., Keresztes, R.S. 1984. Protein-lipid interactions in antipodal plasma membranes of rat colonocytes. *Biochim. Biophys. Acta* **773:**290–300

Bridges, R.J., Rack, M., Rummel, W., Schreiner, J. 1986. Mucosal plexus and electrolyte transport across the rat colonic mucosa. *J. Physiol.* (*London*) **376:**531–542

Fernandez, Y.J., Boigegrain, R.M., Cambon-Gros, C.D., Mitjavala, S.E. 1984. Sensitivity of Na^+-coupled D-glucose uptake, Mg^{2+}-ATPase and sucrase to perturbations of the fluidity of brush-border membrane vesicles induced by *n*-aliphatic alcohols. *Biochim. Biophys. Acta* **770:**171–177

Frizzell, R.A., Heintze, K. 1979. Electrogenic chloride secretion by mammalian colon. *In*: Mechanisms of Intestinal Secretion. H.J. Binder, editor. pp. 101–110. Alan R. Liss, New York

Frizzell, R.A., Rechkemmer, G., Shoemaker, R.L. 1986. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. *Science* **233:**558–560

Giraldez, F., Sepúlveda, F.V., Sheppard, D.N. 1988. A chloride conductance activated by adenosine 3′, 5′-cyclic monophosphate in the apical membrane of *Necturus* enterocytes. *J. Physiol.* (*London*) **395:**597–623

Greger, R., Schlatter, E., Gögelein, H. 1985. Cl^--channels in the apical membrane of the rectal gland "induced" by cAMP. *Pfluegers Arch.* **403:**446–448

Greger, R., Schlatter, E., Gögelein, H. 1987. Chloride channels in the luminal membrane of the rectal gland of the dogfish (*Squalus acanthias*). *Pfluegers Arch.* **409:**114–121

Halm, D.R., Rechkemmer, G.R., Schoumacher, R.A., Frizzell, R.A. 1988. Apical membrane chloride channels in a colonic cell line activated by secretory agonists. *Am. J. Physiol.* **254:**C505–C511

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp technique for high-resolution current recordings from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hayslett, J.P., Gögelein, H., Kunzelmann, K., Greger, R. 1987. Characteristics of apical chloride channels in human colon cells (HT 29). *Pfluegers Arch.* **410:**487–494

Horvath, P.J., Ferriola, P.C., Weiser, M.M., Duffey, M.E. 1986. Localization of chloride secretion in rabbit colon: Inhibition by anthracene-9-carboxylic acid. *Am. J. Physiol.* **250:**G185–G190

Lipkin, M. 1981. Proliferation and differentiation of gastrointestinal cells in normal and disease states. *In*: Physiology of the Gastrointestinal Tract. L.R. Johnson, editor. pp. 145–168. Raven, New York

Lübcke, R., Haag, K., Berger, E., Knauf, H., Gerok, W. 1986. Ion transport in rat proximal colon in vivo. *Am. J. Physiol.* **251:**G132–G139

Nelson, D.J., Tang, J.M., Palmer, L.G. 1984. Single-channel recordings of apical membrane chloride conductance in A6 epithelial cells. *J. Membrane Biol.* **80:**81–89

Perris, A.D. 1965. Isolation of the epithelial cells of the rat small intestine. *Can. J. Biochem.* **44:**687–693

Reinhardt, R., Bridges, R.J., Rummel, W., Lindemann, B. 1987. Properties of an anion-selective channel from rat colonic enterocyte plasma membranes reconstituted into planar phospholipid bilayers. *J. Membrane Biol.* **95:**47–54

Shoemaker, R.L., Frizzell, R.A., Dwyer, T.M., Farley J.M. 1986. Single chloride channel currents from canine tracheal epithelial cells. *Biochim. Biophys. Acta* **858:**235–242

Welsh, M.J. 1986. An apical-membrane chloride channel in human tracheal epithelium. *Science* **232:**1648–1650

Welsh, M.J., Liedtke, C.M. 1986. Chloride and potassium channels in cystic fibrosis airway epithelia. *Nature* (*London*) **322:**467–470

Welsh, M.J., Smith, P.L., Fromm, M., Frizzell, R.A. 1982. Crypts are the site of intestinal fluid and electrolyte secretion. *Science* **218:**1219–1221

Ziomek, C.A., Schulman, S., Edidin, M. 1980. Redistribution of membrane protein in isolated mouse intestinal epithelial cells. *J. Cell Biol.* **86:**849–857

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