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Properties of an Anion-Selective Channel from Rat Colonic Enterocyte Plasma Membranes Reconstituted into Planar Phospholipid Bilayers

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Summary. Vesicles derived from epithelial cells of the colonic mucosa of the rat were fused to planar phospholipid bilayer membranes, revealing spontaneously switching anion-conducting channels of 50 pS conductance (at −30 mV with 200 mM Cl^- each side). The equilibrium selectivity series was I^- (1.7)/Br^- (1.3)/Cl^- (1.0)/F^- (0.4)/HCO_3^- (0.4)/Na (< 0.11). Only one dominant open-state conductance could be resolved, which responded linearly to Cl^- concentrations up to 600 mM. The single-channel current-voltage curve was weakly rectifying with symmetrical solutions. When 50 mV were exceeded at the high-conductance branch of the curve, switching was arrested in the closed state. At more moderate voltages (± 40 mV) kinetics were dominated by one open state of about 35-msec lifetime and two closed states of about 2 and 9-msec lifetime. Of these, the more stable closed state occurred less often. At these voltages one additional closed state of significantly longer lifetime (> 0.5 sec) was observed.

Key Words intestine · epithelial transport · chloride channels · selectivity · inactivation · bilayer reconstitution

Introduction

Secretion of Cl^- across epithelia is thought to occur by a two-step process. The entry of Cl^- into the cell is mediated by an electroneutral, furosemide-sensitive, Na^+-coupled cotransporter in the basolateral membrane (Heintze, Stewart & Frizzell, 1983). As a result of the electrochemical potential gradient for Na^+, maintained by the basolateral Na^+,K^+-ATPase, Cl^- is accumulated within the cell above its electrochemical equilibrium potential (Welsh, Smith & Frizzell, 1982, 1983*a*,*b*; Shorofsky, Field & Fozzard, 1984). Chloride exits the cell by passive diffusion across the apical membrane. Agents that stimulate Cl^- secretion act by increasing the apical membrane conductance for Cl^- (e.g., Klyce & Wong, 1977; Welsh et al., 1982; Welsh, 1986).

This model for Cl^- secretion implies the existence of a regulated apical membrane entity that can conduct chloride ions. For the colon, the transport properties of this putative channel have, as yet, remained undescribed. Here we report some properties of an anion-selective channel that may be the apical membrane Cl^- channel. Our results appeared in abstract form (Reinhardt et al., 1986).

Materials and Methods

Isolation of Membrane Vesicles

Female Sprague-Dawley rats approximately 250 g in weight were used. They had free access to food (Altromin Diet No. 1320, Lage, F.R.G) and water until the time of the experiment. For reasons not directly related to this study the animals were injected subcutaneously 66, 42 and 18 hr before the isolation of the colon with dexamethasone (6 mg kg^{-1} suspended in corn oil). This pretreatment causes the expression of amiloride-sensitive Na^+ channels in the apical membrane but has no effect on the capacity of the rat colon to secrete Cl^-, as measured in Ussing chambers under short-circuit conditions. The appearance of Na channels is useful for identification of apical membrane material.

The muscularis propria and submucosa were removed from the colon *descendens* as described by Andres et al. (1985). The distal portion of the colonic mucosa was mounted as a flat sheet on a small plastic holder that had a 3-cm^2 orifice. The tissue was then immersed in a warm (37°C) solution containing (mM): 10 EDTA, 107 NaCl, 25 $NaHCO_3$, 4.5 KCl, 1.8 Na_2HPO_4, 0.2 NaH_2PO_4 and 12 glucose. The solution was gassed with 5% CO_2 and had a pH of 7.4. Every 5 min, for 30 to 40 min, the tissue and holder were attached to a mechanical vibrator and vibrated for 15 sec in a cold (4°C) solution of 100 mM K_2SO_4, 50 mM Tris-H_2SO_4, 5 mM EGTA, pH 7.4, and then returned to the EDTA-containing solution. One piece of tissue from each of four to eight rats was prepared at one time. Histological studies revealed that all of the epithelial cells were removed by this treatment and that the underlying connective tissue was completely intact (Bridges et al., 1986).

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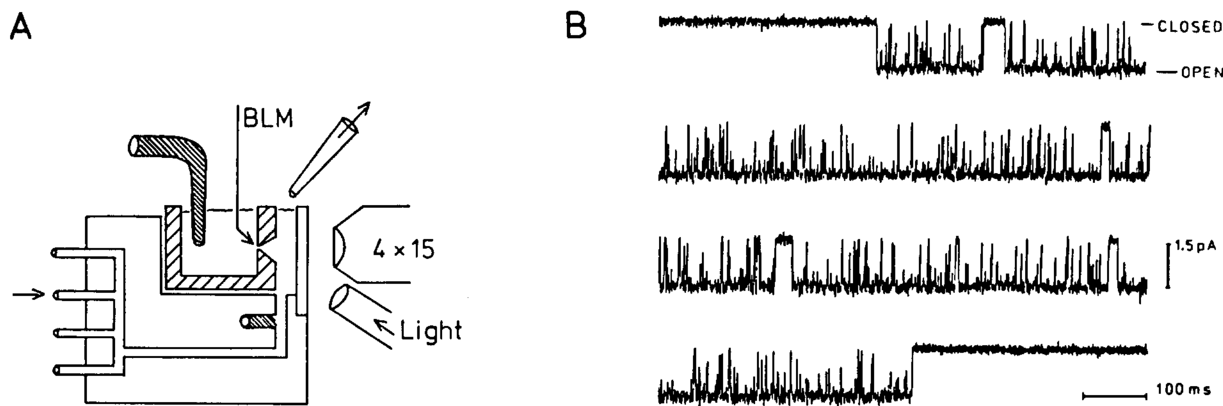


Fig. 1. (*A*) Schematic cross section through the experimental chamber constructed to change the *cis* solution of the phospholipid bilayer by perfusion. The body of the chamber was made of Lucite® but the cylindrical inset containing the *trans* solution (250 μl volume) and the orifice (arrow) of Lexan (TKG W. Ensinger, Nufringen, FRG). Illumination of the black lipid membrane (BLM) with a light guide and observation with a horizontal microscope (4 times 15 ×) were done through a glass window cut from a microscopy slide. The volume of the *cis* compartment was 150 μl. Fifteen solutions could be connected to perfusion ports as those shown on the left. Flow through the *cis* compartment was controlled with hemostat clamps and excess solutions removed with a water suction pump. Electrodes were connected through agar bridges (3 M KCl), which are indicated by dense hatching. (*B*) Spontaneous switching behavior of a reconstituted chloride channel exposed to symmetrical 200 mM NaCl solutions and −30 mV (*cis* versus *trans*). Negative single-channel currents, corresponding to Cl^- movement from *cis* to *trans*, are plotted downward. The record comprises 3 sec. For noise suppression, the plotting bandwidth was reduced to 90 Hz

The cell suspension, consisting of only epithelial cells, was centrifuged for 5 min at 300 × *g*. This and all subsequent steps in the membrane isolation were at 4°C. The volumes given are for cells collected from two colons. The cells were washed 3 times with 30 ml of 8.5% sucrose, 5 mM histidine-imidazole, 1 mM EGTA, pH 7.4, sucrose buffer solution. They were then homogenized (20 strokes) with a tight-fitting Dounce-type homogenizer in 20 ml of the same solution. The homogenate was centrifuged at 1000 × *g* for 10 min and the supernatant collected. This was repeated three times. The pooled supernatant was centrifuged at 20,000 × *g* for one hr. The outer white fluffy portion of the pellet was collected, suspended in 1.5 ml sucrose buffer and homogenized (5 strokes) with a loose-fitting Dounce-type homogenizer. This was placed on top of a discontinuous sucrose density gradient consisting of three ml each of 20, 40 and 60% sucrose (wt/wt) buffered with 5 mM histidine-imidazole, pH 7.4, and centrifuged in a Beckman SW Ti 40 rotor at 130,000 × *g* for 90 min. The 20/40 interface was collected, diluted with 30 ml sucrose buffer and centrifuged at 27,000 × *g* for 1 hr. The pellet was resuspended in 0.5 ml sucrose buffer and kept frozen at −20°C.

In the final membrane vesicle fraction approximately 0.3 mg protein was recovered per rat colon. This corresponded to 1 to 3% of the protein in the initial homogenate. Enzyme marker studies demonstrated a 10- to 15-fold enrichment in the final membrane fraction compared to the initial homogenate for the plasma membrane marker enzymes alkaline-phosphatase and Na^+,K^+-ATPase. In addition the ratios of the final membrane fraction to the initial homogenate in the specific activities of succinate dehydrogenase, a mitochondrial enzyme marker, and NADPH-cytochrome *c* reductase, an endoplasmic reticulum enzyme marker, were less than 0.1.

Incorporation of Channels into Planar Bilayers

Phospholipid bilayers were raised in a chamber designed for easy exchange of the *cis* solutions (Fig. 1*A*). The orifice diameter was 150 μm. A 2% solution of phosphatidylethanolamine (Avanti Polar Lipids) in *n*-decane was painted onto this opening from the *trans* side. Bilayer formation was observed optically, using a horizontally mounted microscope (magnification 60×), and electrically by observing the increase in membrane capacitance which accompanies thinning.

Electrodes were Ag-$AgCl_2$ pellets immersed in 3 M KCl and connected to the solutions through 3 M KCl agar bridges. The gravity-perfused *cis* solution was grounded and the *trans* solution potential was controlled by the current-recording amplifier, which had a feedback resistor of 1 GΩ and a bandwidth of 2 kHz. The otherwise unfiltered amplified current and voltage signals were digitized (16 bit, 40 kHz) with an audio PCM processor (Sony 501 ES) modified to pass zero frequency, and stored on a videotape recorder. For previewing and playback of the records a 4-pole Butterworth anti-aliasing filter of adjustable cutoff was used (200 or 250 Hz for the Table and Fig. 4). Kinetic analysis of the records was done by computer.

The *trans* solution was typically 200 mM NaCl containing 1 mM EGTA and 2 mM HEPES buffer adjusted to pH 7.0 with NaOH. Bilayers were formed using the same solution on the *cis* side. The *cis* solution was exchanged for one of 600 mM NaCl prior to addition of vesicles to the *cis* compartment. Five μl of vesicle suspension were usually sufficient. When pipetted directly before the bilayer, this amount yielded fusion events typically within the first minute. Fusion rate was prohibitively low when the *cis* NaCl concentration was lowered to 200 mM. Fusion was detected by the appearance of switching events on the current record. Excess vesicles were then washed away and the NaCl concentration lowered by perfusing the *cis* half-chamber (150 μl volume) at 16 ml min^{-1} for 1.5 min or longer. This perfusion rate introduced some microphonic current noise, but the channel activity remained recognizable on the oscilloscope. Records were taken while perfusion was stopped.

For the solutions used, calculated and measured electrode offset potentials were smaller than 1 mV. Membrane voltages are referenced to the *trans* solution and current from *cis* to *trans* is designated positive. As fusion occurred from the *cis* side, posi-

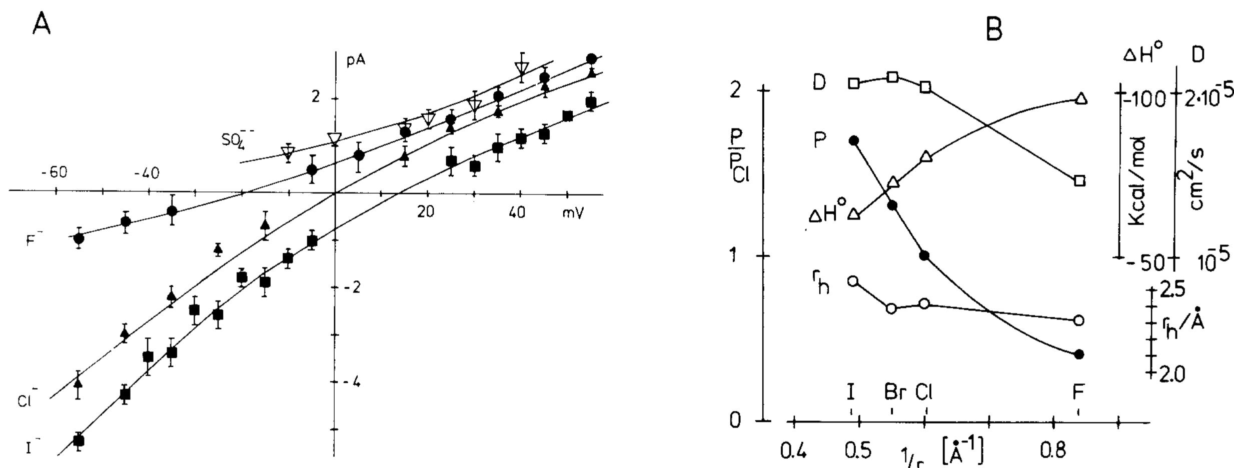


Fig. 2. (*A*) Voltage dependence of single-channel currents recorded with 200 mM NaCl in the *trans* solution and 200 mM of various Na salts (parameter) in the *cis* solution. The *trans* potential is reference and *cis* → *trans* current is positive. The sulfate concentration used was close to 200 mval liter^{-1}. Observations at voltages more negative than −55 mV were not possible because of inactivation. Data points are means of 10 to 30 measurements; bars indicate SEM; lines drawn by eye. (*B*) The relative permeabilities (P/P_{Cl}), as calculated from reversal potentials, were plotted against the inverse crystal radius of the permeant halide anions. Also plotted were the diffusion coefficient in water (*D*), the standard entropy of hydration (ΔH°) and the radius of ion plus "primary" hydration shell (r_h) as listed by Edwards (1982) and Hille (1984), respectively

tive voltages indicate a polarization of the channel's diffusion pathway corresponding to vesicle negative with respect to outside solution.

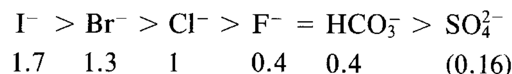
Results

With symmetrical NaCl concentrations of 200 mM and a membrane voltage of −30 mV (*cis* versus *trans*) the channel exhibited spontaneous switching behavior as shown in Fig. 1*B*. For these conditions, the resolved current step amplitudes of 1.5 pA indicate a dominant single-channel chord conductance of 50 pS. As the signal-to-noise ratio required heavy filtering, sub-levels of this conductance could not be resolved. However, if such sub-levels exist their lifetime must be short.

Current amplitudes changed nonlinearly with voltage as shown in Fig. 2*A*. Similar rectification was reported for the tracheal apical Cl^- channel (e.g. Welsh, 1986). When assuming that the rectification is of same sign in the colonic and tracheal channel, the colonic channels were oriented in the bilayer with the cellular side facing the vesicle interior and the *trans* solution, respectively.

The Cl^-/Na^+ selectivity could be roughly estimated from the reversal potentials of the *I*(*V*) curves, which were corrected for electrode offset potentials. Using 300 mM NaCl on the *trans* side and 600 mM on the *cis* side, one expects a reversal potential of 17.9 mV for an ideally anion-selective channel. Values obtained in four experiments were 14.3, 15, 16 and 18 mV. The least favorable of these values implies a Cl^-/Na^+ selectivity of 9.3. We may conclude, therefore, that the Cl^-/Na^+selectivity was 10 or larger. The mean of the reversal potentials indicates a selectivity ratio of 16.

When the *cis* chloride was replaced by 200 mM iodide, the *trans* → *cis* current became more negative, indicating that I^- is more permeable than Cl^-. Similar substitutions with other halide anions, bicarbonate and sulfate yielded the equilibrium selectivity series



$$\begin{array}{cccccc} I^- > & Br^- > & Cl^- > & F^- = & HCO_3^- > & SO_4^{2-} \\ 1.7 & 1.3 & 1 & 0.4 & 0.4 & (0.16) \end{array}$$

as calculated from the biionic reversal potentials (Fig. 2*A*), which were also corrected for electrode offset potentials. A Na^+/Cl^- selectivity of 0.063 was used in this calculation. While HCO_3^- currents where obtained at a *cis* pH of 8.2 rather than 7.0, control experiments showed that Cl^- currents were not affected by changing the pH from 7.0 to 8.2. The value of 0.16 given for the relative SO_4^{2-} permeability was estimated from the SO_4^{2-} and Cl^- currents at −10 mV, because the reversal potential was not observed in this case.

The equilibrium permeabilities follow Eisenman's halide anion sequence I, i.e., they increase linearly with the crystal radius and with decreasing hydration enthalpy of the ion (Fig. 2*B*). A permeability optimum was not found, nor is it expected for sequence I (*see* Fig. 4 in Eisenman and Horn, 1983). When permeability ratios were calculated from the currents observed at different voltages

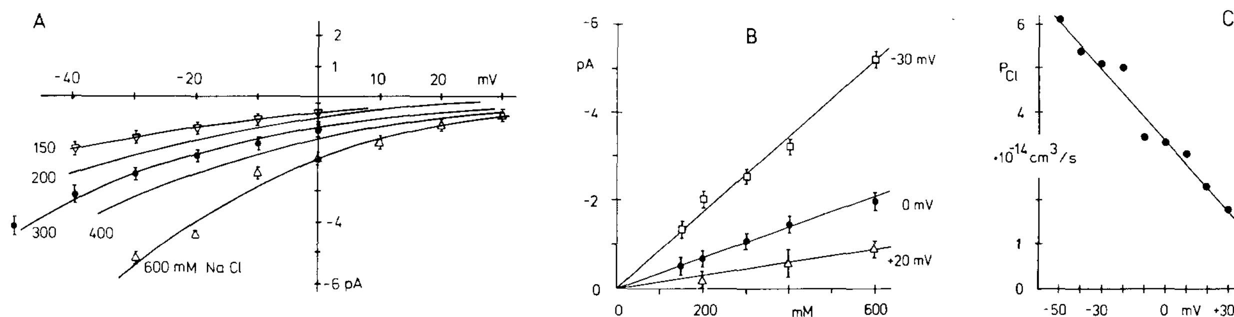


Fig. 3. (*A*) Voltage dependence of single-channel chloride currents obtained with 1 mM NaCl plus 199 mM Na gluconate in the *trans* solution and NaCl of various concentrations (parameter) in the *cis* solution. Means and SD for three concentrations were plotted; points for 200 and 400 mM NaCl were left out for graphical clarity. Lines drawn by eye. (*B*) Concentration dependence of single-channel chloride currents, obtained as described for panel *A*. The nonsaturating relationship was plotted for three membrane voltages, as indicated. (*C*) Voltage dependence of chloride permeability, calculated with the GHK-current equation for the experiment of panels *A* or *B*

(Fig. 2*A*), the same selectivity sequence was found, but the permeability ratios varied with voltage. P_F/P_{Cl} changed from 0.3 at −45 mV to 0.5 at +45 mV, and P_I/P_{Cl} from 1.3 at −45 mV to 3 at +45 mV. It appears, therefore, that under the conditions used halide permeabilities depend on voltage to different degrees, that of I^- changing more than that of F^-.

When the chloride concentration of the *trans* solution was lowered from 200 to 1 mM by replacement with gluconate, the single-channel conductance, as determined at 0 mV, decreased to roughly half the previous value, indicating that gluconate was nonpermeant and did not block strongly from the *trans* side. Variation of the *cis* NaCl concentration from 150 to 600 mM then yielded the set of current-voltage curves shown in Fig. 3*A*. In the voltage range investigated (−50 to +30 mV) the single-channel current did not show significant saturation up to 600 mM Cl^- on the *cis* side (Fig. 3*B*). Linearity of this relationship prevailed at all voltages of this range. From the slope obtained at 0 mV we calculate, using the GHK-current equation, an open-channel permeability for Cl^- of $3.3 \cdot 10^{-14}$ cm^3 sec^{-1}, and a voltage dependence of $-5.6 \cdot 10^{-16}$ cm^3 sec^{-1} mV^{-1} (Fig. 3*C*).

At −45, −30 and +35 mV the switching kinetics were analyzed for symmetrical solutions of 200 mM NaCl. The fitting of open-time histograms (e.g. Fig. 4*A*) with sums of exponentials revealed a single mean open time of 30 msec at −45 mV and of 41 msec at −30 mV, while at +35 mV two mean open times, of 2 and 41 msec, were retrieved from the records. However, the longer lived of the two open states remained dominant, having a 20-fold larger probability of occurrence. From shut-time histograms (e.g. Fig. 4*B*), two closed states with short lifetimes (about 2 and 9 msec) were found, which also were only weakly dependent on voltage. Here, however, the more labile state had a 2 to 7 times larger probability of occurrence than the more stable one. In addition, there exists at least one long-lived closed state. To give an example, at +35 mV (Fig. 4*B*) 28 shut periods longer than 50 msec were recorded, but necessarily excluded from statistical analysis in the msec range. Their mean duration (± SEM) was 1.2 ± 0.21 sec. The kinetic results obtained for *short* lifetimes are summarized in the Table.

When the *cis* solution was made more negative than −50 mV with respect to *trans*, opening events became infrequent, the channel being arrested in the closed state for long periods of time. It may be speculated that this is due to an increase in the lifetime of the long-lived closed state at negative voltages. By way of example, Fig. 5 shows that on switching from 0 to −55 mV the channel remained open for less than 1 sec, then closed for 28 sec. In this period the voltage was increased up to +20 mV without causing a single opening. However, on changing to +30 mV the channel remained closed for only 1 sec, then resumed open/close switching for the next 12 sec (after which time observation was terminated).

Discussion

The single-channel conductance of 50 pS obtained for the anion-selective channel from rat colonic enterocyte plasma membranes is similar to that of *apical* anion channels from the dogfish rectal gland (Greger, Schlatter & Gögelein, 1985) and the canine (Shoemaker et al., 1986; Welsh, 1986) and the human trachea (Frizzell, Rechkemper & Shoemaker, 1986). These epithelia, like the rat colon, secrete chloride in response to stimuli which increase the

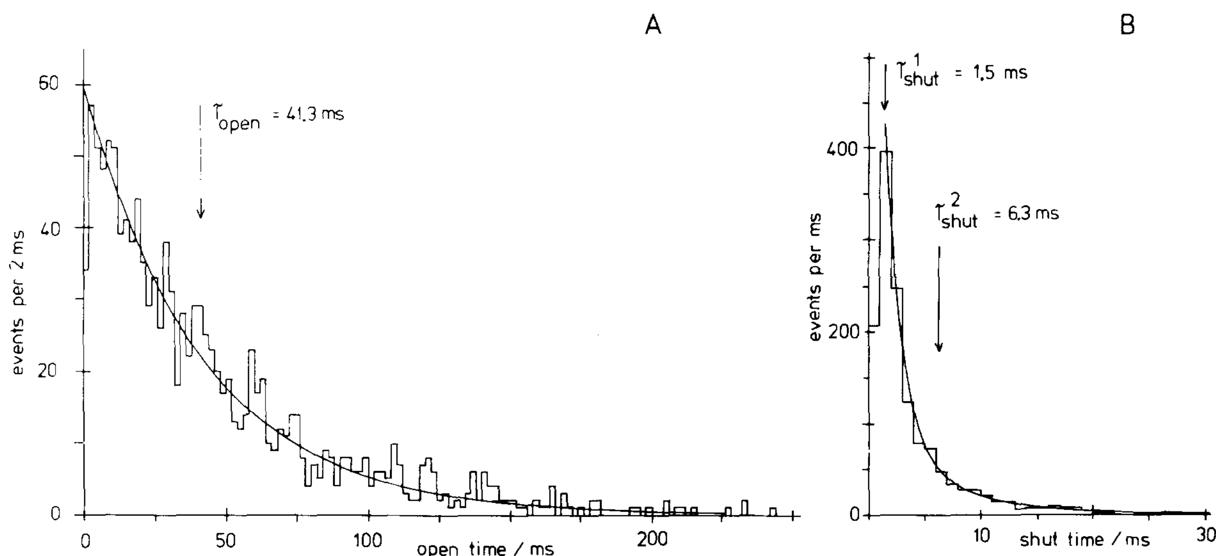


Fig. 4. (*A*) Open-time histogram of spontaneous switching of a chloride channel recorded for 180 sec at −30 mV with symmetrical solutions of 200 mM NaCl. Anti-aliasis filter 200 Hz. Dwell time 0.25 mec, binwidth 2 msec, 1283 events (all) included. (*B*) Shut-time histogram obtained from a record of 380 sec at +35 mV with symmetrical solutions of 200 mM NaCl. Anti-aliasis filter 250 Hz. Dwell time 0.25 msec, binwidth 1 msec, 2888 events included, 28 shut periods lasting 1.2 ± 0.21 sec (mean ± SEM) were excluded. Judged by the integral of each of the two exponentials, the faster opening events occurred at +35 mV with a probability of 2.3 times larger than the probability of the slower opening events. However, at less positive voltages, the faster opening events were more dominant (Table)

cellular concentration of cAMP. The weakly rectifying single-channel $I(V)$ curve (Figs. 2*A* and 3*A*) was also observed with *apical* excised patches of the trachea (Frizzell et al., 1986; Shoemaker et al., 1986; Welsh, 1986).

Single-channel conductances of greater than 350 pS have been reported for anion channels from *apical* membranes of A6 kidney epithelial cells (Nelson, Tang & Palmer, 1984) and pulmonary alveolar (type II) cells (Schneider et al., 1985), as well as *basolateral* membranes from surface cells of mammalian urinary bladders (Hanrahan, Alles & Lewis, 1985). However, these three types of channels were reported to have sub-states of lower conductance. The channel from pulmonary epithelial cells adopts six different open states that are integer multiples of 60 to 70 pS (Krouse, Schneider & Gage, 1986), a value close to that of the low-conductance channels mentioned above. It has been suggested that the pulmonary epithelial anion channels consists of six conductive pathways, "co-channels," in parallel (Krouse et al., 1986). A "double-barreled" anion channel has been reported for the plasma membranes of the electric organ of *Torpedo californica* (Hanke & Miller, 1983; Miller & White, 1984). In the colonic anion channel we only resolved the dominant open-state conductance, but no short-lived substate conductances. Future studies must decide whether this is the only open-state conductance for this channel.

Table. Kinetic results obtained from spontaneously switching chloride channels at three voltages[a]

mV	τ (open)/msec		τ (shut)/msec	
	1	2	1	2
−45	—	30. (150)[b]	2.25 (1200)	7.5 (45)
−30	—	41.25 (60)	1.4 (1250)	12.5 (20)
+35	2 (150)	41.25 (135)	1.5 (1950)	6.25 (200)

[a] The decay time constants of channel states of *short* lifetime, as obtained from duration histograms (e.g. Fig. 4), are listed.
[b] Numbers in brackets are exponential amplitudes (events/sec) obtained by retropolation to zero time.

The channel currents did not saturate up to 600 mM Cl^-. Therefore, the channel occupancy will be low and it is not surprising that the equilibrium selectivity *sequence* (obtained from reversal potentials) is identical with the sequence obtained from currents or conductances. The selectivity of this channel appears to change with voltage, but remains small in the ±50 mV range used.

The equilibrium selectivity sequence of the colonic channel for the halide anions is $I^- > Br^- > Cl^- > F^-$, i.e. the ion of largest crystal radius passes the channel most easily. Therefore, simple size discrim-

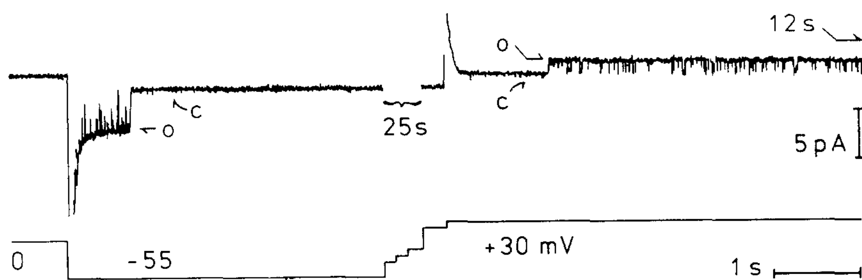


Fig. 5. Reversible inactivation of what appears to be bursting activity, observed at holding voltages more negative than −50 mV (*cis* versus *trans*). Both solutions 200 mM NaCl. The channel states *open* (*o*) and *closed* (*c*) are indicated. After closing at −55 mV, the channel remained closed for 28 sec, even though the voltage was increased up to +20 mV. At +30 mV the channel resumed switching activity for 12 sec, then the voltage was changed again

ination based on the crystal radius or on the radius which includes the primary hydration shell (Fig. 2*B*) does not seem to be essential for these ions, although it may play a role in the rejection of SO_4^{2-} and gluconate ions. The same sequence and similar relative permeabilities were observed in the channel from pulmonary epithelial cells (Schneider et al., 1985). It is sequence I of Eisenman's seven halide anion sequences (e.g. Diamond & Wright, 1969; Wright & Diamond, 1977).

The simultaneous presence of two permeant species is obviously required for the measurement of equilibrium selectivity. This parameter, according to established concepts, merely reflects differences in peak heights of activation energies, but not differences in binding of the two species in the channel (e.g. Bezanilla & Armstrong, 1972; Hille, 1975), provided the channel is not occupied by more than one ion at a time (Eisenman & Horn, 1983). This is intuitively understandable, because when two species compete for a binding site, stronger binding of one will lower the transport rates of both. Thus sequence I indicates that the permeant ion of largest radius has the smallest energy of activation in terms of peak heights. Part of this energy may be required for partial stripping of the hydration shell as the ion enters the selectivity filter. Indeed, due to its smaller field strength the larger ion has the smaller heat of hydration (Fig. 2*B*). Thus I^- may pass the channel faster than Cl^- because Cl^- is more firmly hydrated in free solution. Furthermore, in order for the hydration/dehydration process to become rate limiting, other processes like coulombic interaction with the filter structure must be small, i.e., contribute little to the height of the filter's energy peak.

The Cl/Na selectivity may be based on charge discrimination. The pulmonary channel has a Cl/Na selectivity ratio of 67, while our data for the colonic channel indicate ratios > 10 without, as yet, yielding a definite value. The anion channel from A6 kidney epithelial cells has a Cl/Na selectivity no larger than nine, and a Cl/SO_4 selectivity of 0.5 (Nelson et al., 1984). That of the colonic channel is about 0.16.

While I^- is not secreted transmurally by the rat colon, the short-circuit current remains essentially unchanged when Br^- is substituted for Cl^-, indicating that Br^- can pass both membranes. (F^-, because it is toxic, was not tested; Bridges & Schreiner, *unpublished observations*.) These differences between the ion selectivity for transepithelial anion movement and the single-channel selectivity are likely to be due to the selectivity of the entry step at the basolateral membrane. Thus one might predict that the Na-coupled cotransporter on the basolateral membrane, which mediates the entry of Cl^-, transports Cl^- and Br^- at similar rates but is unable to transport I^-, or does so at a much slower rate.

Arrest of gating activity at large negative voltages (Fig. 5) was seen regularly with the reconstituted Cl^- channel. At present the exact relationship between closed-state lifetime and voltage near −50 mV cannot be specified, because records were generally not long enough to retrieve lifetimes in the order of 10 sec with statistical significance. Pronounced voltage-dependent activation is known from other Cl^--transporting systems. For instance, the apical Cl^- channel of toad skin activates when the cellular potential is made positive relative to the outside solution (e.g. Larsen & Rasmussen, 1982). Similarly, Cl^- channels of skeletal muscle open more frequently during depolarization (e.g. Blatz & Magleby, 1985). For the colonic Cl^- channel, the functional significance of voltage-dependent inactivation remains to be elucidated. It will be important to ascertain that the functional channel on the cell membrane shares this property with the reconstitutioned channel, as it has become known that Cl^-

channels can change their gating behavior when contact to the cytosol is lost (Bosma, 1986).

A salient question to be addressed is whether this anion channel is the apical membrane channel involved in Cl^- secretion. As yet we did not undertake to separate apical membranes from basolateral membranes. The basolateral membrane of Cl^--secreting epithelia is, however, reported to have a low conductance for Cl^- (Welsh et al., 1982). Uptake studies with the same membrane vesicles used in this study have demonstrated that more than 90% of the uptake of ^{22}Na is inhibited by 1 μM amiloride (Bridges, Garty & Rummel, *in preparation*). A cation-selective, amiloride-sensitive channel was repeatedly observed in our bilayer experiments (Reinhardt, Bridges, Rummel & Lindemann, *unpublished observations*). This channel is probably the best marker protein for apical membrane material from dexamethasone-pretreated colonic cells presently available. Occasionally, both a cation and anion channel were present simultaneously in the bilayer and by appropriate switching of the solutions each could be studied separately.

These observations show that apical material was present in the 20/40 vesicle fraction used in this study and suggest that the anion-selective channel described may be derived from the apical membrane. The discussed similarities of the colonic channel to apical Cl^- channels of other Cl^--secreting epithelia also point to an apical origin. However, we cannot exclude contamination of the 20/40 fraction used with basolateral membrane material. Thus future studies will have to deal more directly with the origin of this anion channel and its putative role in the Cl^- secretory process of the colon mucosa.

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