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cAMP-Activated Chloride Channel in the Basolateral Membrane of the Thick Ascending Limb of the Mouse Kidney

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Summary. The properties of an anion-selective channel observed in basolateral membranes of microdissected, collagenase-treated, cortical thick ascending limbs of Henle's loop from mouse kidney were investigated using patch-clamp single-channel recording techniques. In basal conditions, single Cl^- currents were detected in 8% of cell-attached and excised, inside-out, membrane patches whereas they were observed in 24% of cell-attached and 67% of inside-out membrane patches when tubular fragments were preincubated with Forskolin (10^{-5} M) or 8-bromo-cAMP (10^{-4} M) and isobutylmethylxanthine (10^{-5} M). The channel exhibited a linear current-voltage relationship with conductances of about 40 pS in both cell-attached and cell-free membrane configurations. A P_{Na^+}/P_{Cl^-} ratio of 0.05 was estimated in the presence of a 142/42 mM NaCl concentration gradient applied to inside-out membrane patches. Anionic selectivity of the channel followed the sequence $Cl^- > Br^- > NO_3^- \gg F^-$; gluconate was not a permeant species. The open-state probability of the channel increased with membrane depolarization in cell-attached, i.e., *in situ* membrane patches. In excised, inside-out, membrane patches, the channel was predominantly open with the open-state probability close to 0.8 over the whole range of potentials tested (−60 to +60 mV). The channel activity was not a function of internal calcium concentration between 10^{-9} and 10^{-3} M. We suggest that this Cl^- channel, whose properties are distinct from those in other epithelia, could account for the well-documented conductance which mediates Cl^- exit in the basolateral step of NaCl absorption in thick ascending limb of Henle's loop.

Key Words Cl^- channel · cAMP · kidney · patch clamp

Introduction

The thick ascending limb of Henle's loop reabsorbs sodium chloride from the lumen of the kidney tubule; in particular, transmural chloride transport occurs against a transepithelial electrochemical potential. This region of the nephron is a target for several hormones which act via the second messenger, cAMP (Morel, Imbert-Teboul & Chabardès, 1981). Since the development of the in vitro single-tubule microperfusion technique (Burg et al., 1966), a model for NaCl absorption across the thick ascending limb of Henle's loop has progressively emerged. In its simplest form, this model includes (*see* Greger, 1988) a Na^+-K^+-$2Cl^-$ cotransport system at the apical membrane which brings Na^+ and Cl^- ions into the cell (in some species, including the mouse, synchronous apical Na^+/H^+ and Cl^-/HCO_3^- exchangers are also present; Friedman & Andreoli, 1982) while K^+ ions are recycled back into the lumen across the apical membrane through a diffusion pathway. Na^+ ions are removed from the cell by a basolateral Na^+-K^+ pump and a basolateral Cl^- conductance allows Cl^- diffusion across the peritubular membrane. While elementary ion transport systems may differ from one tissue to another, several NaCl-absorbing and Cl^--secreting epithelia nonetheless share a common principle for Cl^- transfer with the thick ascending limb of Henle's loop. This consists of a neutral NaCl-transporting device in series with a Cl^- conductance; polarity of transport is then determined by the location of the various transport systems (O'Grady, Palfrey & Field, 1987).

Although the Cl^- conductance at the basolateral membrane of the thick ascending limb of Henle's loop is well documented (Greger & Schlatter, 1983; Schlatter & Greger, 1985; Yoshitomi et al., 1987), no published information is available on the individual Cl^- channel(s) which underly the macroscopic Cl^- conductance. The present study describes the novel properties of an anion-selective channel in the basolateral membrane of the cortical thick ascending limb of Henle's loop of the mouse kidney. The channel appears to have properties quite distinct from other epithelial Cl^- channels described to date, which include the intensively studied outwardly rectifying Cl^- channel found in several epithelia (Frizzell, Rechkemmer & Shoemaker, 1986*b*; Welsh, 1986*b*; Hayslett et al.,

1987; Halm et al., 1988) and more recently in lymphocytes (Chen, Schulman & Gardner, 1989) and fibroblasts (Bear, 1988).

Materials and Methods

Tissue Preparation

Cortical thick ascending limbs of Henle's loop (cTALH) were isolated from the kidneys of male mice (15–20 g) as previously described (Teulon, Paulais & Bouthier, 1987). Briefly, one kidney was perfused via the renal vein with NaCl-Ringer solution (pH 7.4, 1 mM $CaCl_2$, *see below* for composition) containing 200–400 U/ml collagenase (Worthington CLSPA). Small cortical-medullary slices of kidney were cut and incubated in the same collagenase-supplemented solution. Incubation times (30–60 min) and temperatures (30–37°C) were adjusted to find the best combination for removal of the basement membrane covering the basolateral plasma membrane in each animal. cTALH fragments were dissected out under a stereomicroscope with the tissue immersed in NaCl-Ringer solution (pH 7.4, 10^{-4} M $CaCl_2$, 0.8 mM NaH_2PO_4, 4 mM Na_2HPO_4) at 4°C.

Patch-Clamp Recording Conditions

A fragment of cTALH tubule was transferred to a chamber on the stage of an inverted microscope (IM Carl Zeiss, D-7082, Oberkochen, Germany). Single-channel currents were recorded with an RK300 amplifier (Biologic, Echirolles, France) from patches of basolateral membranes of cTALH fragments using the cell-attached and inside-out cell-free variants of the patch-clamp technique (Hamill et al., 1981). Patch pipettes were made from hematocrit capillary tubes (CHR, Badram, Bizkerod, Denmark) pulled in two stages with a M720 pipette puller (David Kopf Instruments, Tujunga, CA) and coated with a silicone elastomer (Sylgard, Dow Corning, Seneffe, Belgium). Data were stored on a video tape after analog-digital conversion by a pulse-code-modulation device (Sony PCM 701, Biologic) and played back onto the oscilloscope screen (Tektronix, Beaserton, OR), or through a 902LPF 8-pole Bessel low-pass filter (Frequency Devices, Haverhill, MA) at 0.4–1 kHz.

The bath reference electrode was a 0.5 M KCl (in 4% agar) bridge connected to an Ag-AgCl half-cell. Liquid-junction potentials arising at the bath reference were evaluated with a pipette containing 2.7 M KCl by measuring the zero-current voltage deflections induced by NaCl-substituted solutions.

All electrical potential differences are expressed with respect to the pipette interior; positive (upward) single-channel currents correspond to a cation flux from the bath into the pipette. The experiments were conducted at room temperature (20–30°C).

Data Analysis

Single-channel current recordings were analyzed off-line with an automated single-channel analysis program (IPROC 2, Axon Instruments, Burlingame, CA) running on an IBM AT-compatible microcomputer. Data recorded over 20 to 120 sec were pre-filtered at 1 kHz and digitized at 2–4 kHz using an analog-digital interface (Data translation, Marlborough, MA). The total times that one to N channels were open simultaneously (t_1 to t_N) were measured sequentially; the open-state probability of the channel (P_o) was then calculated from $(\sum_{x=1}^{N} xt)/NT$ where T is the total duration of the recording and N the maximum number of channels seen in the patch. In this study, N never exceeded three.

Relative ion permeabilities were estimated from the reversal potential for current flow (E_r) using the Goldman-Hodgkin-Katz equation without correction for ionic activities.

Experimental values are given as mean ± SEM, n denotes the number of results.

Solutions

At the beginning of each experiment, the cTALH fragment was immersed in a Ringer solution containing (in mM): NaCl, 140; KCl, 4.8; $MgCl_2$, 1.2; $CaCl_2$, 0.01 or 1; HEPES, 10; glucose, 10; adjusted to pH 7.4 with NaOH. The same solution was present in the recording pipette where pH was adjusted to pH 7.2 and the calcium concentration usually reduced to 10^{-9} M by the inclusion of 2 mM EGTA. On formation of an inside-out patch, the recording pipette was perfused with a stream of solution from one of a series of piped outlets. The control solution was a Ringer solution at pH 7.2 with calcium concentration reduced to 10^{-7} M (unless otherwise stated). The anion *vs.* cation selectivity of the channel was investigated with a low NaCl solution containing 42 mM NaCl, 200 mM saccharose and no KCl. Ion selectivity was evaluated by replacing all but 10 mM of NaCl with a different anion salt (nitrate, fluoride, bromide, gluconate or iodide), keeping KCl, $MgCl_2$, and $CaCl_2$ at their control concentrations.

Ca^{2+} concentration below 10^{-5} M was adjusted by adding $CaCl_2$ and EGTA (Teulon et al., 1987). The free calcium concentration was calculated by an iterative method (Fabiato & Fabiato, 1979) using stability constants for all reactions between Ca^{2+}, Mg^{2+}, H^+ and EGTA (Fabiato, 1981).

Results

Channel Activation

Cl^- channel activity was recorded in three of 35 cell-attached membrane patches (8%) when the cTALH fragments were bathed in NaCl-Ringer and clamped at a variety of positive (depolarizing) or negative (hyperpolarizing) voltage values. No further Cl^- channels were activated after excision of the patches from the cells. Since a Cl^- channel observed in tracheal cells (Schoumacher et al., 1987; Li et al., 1988) and lymphocytes (Chen, Schulman & Gardner, 1989) may be activated by depolarizing voltages, we tested the effects of a sustained membrane depolarization on a number of inside-out membrane patches. Depolarizing voltages (from +50 to +100 mV) applied for a total of 5 to 9 min, did not lead to the activation of Cl^- channels in any of nine preparations.

When cTALH fragments were preincubated with 8-bromo-cAMP (10^{-4} M) and isobutylmethyl-

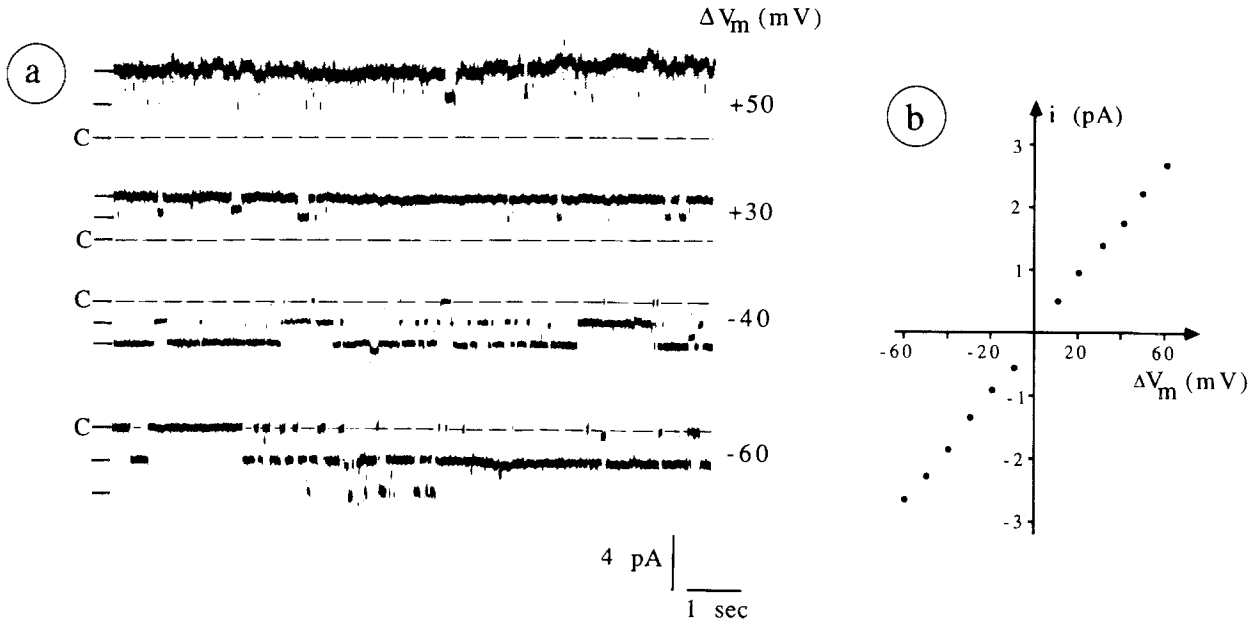

Fig. 1. Single-channel records and current-voltage relationship for one cell-attached membrane patch. The patch pipette and the bath contained NaCl-Ringer solution with Ca^{2+} concentrations of 10^{-9} and 10^{-5} M, respectively. The transmembrane potential across the patch corresponds to the spontaneous resting cell potential for zero change in the patch potential (ΔV_m). The patch membranes are depolarized or hyperpolarized from the spontaneous resting potential for positive or negative ΔV_m, respectively. (*a*) Representative single-channel record traces are shown with the corresponding ΔV_m next to each trace. Outward membrane currents are upwards. Records were filtered at 800 Hz low pass. (*b*) Current-voltage relationship of the channel currents shown in *a*. The single-channel current (*i*) is positive for outward current

xanthine (IBMX) (10^{-5} M) or Forskolin (10^{-5} M) for at least 10 min, we observed single Cl^- currents in 15 of 63 cell-attached membrane patches (24%). As an example Fig. 1*a* shows a typical recording from a cell-attached patch on a cTALH fragment bathed in NaCl-Ringer containing 10^{-5} M Forskolin (recordings with the other two compounds were similar). A NaCl-Ringer solution was present in the recording pipette. The *I-V* relationship recorded from a number of such patches was linear (*e.g.*, Fig. 1*b*), with a slope conductance of 42.5 ± 1.8 pS (n = 15) and a reversal potential close to zero (1.9 ± 1.7 mV). We found that the open-state probability (P_o) of the channel increased with membrane depolarization in cell-attached, i.e., *in situ* membrane patches (four preparations): P_o increased from about 0.4 at −60 mV (the patch membrane being hyperpolarized by 60 mV from the resting membrane potential) to 0.9 at +60 mV (the patch membrane being depolarized by 60 mV) (Fig. 2). With cTALH fragments preincubated in the presence of Forskolin or 8-bromo-cAMP and IBMX, we frequently observed Cl^- channel openings in membrane patches excised from cells where no channel had been recorded in cell-attached mode (27 of the 48 silent patches or 43% of total number of patches). No differences were observed between channels which opened upon patch excision and those which were active in the intact cells. Thus, all told, single Cl^- currents were recorded from 67% of all patches.

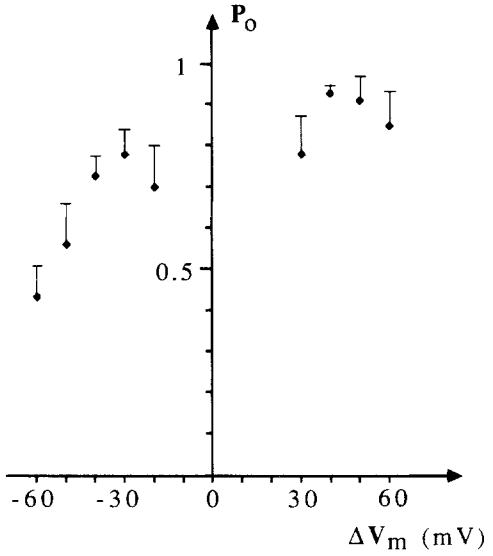

Fig. 2. Effect of patch potential (ΔV_m) upon the open-state probability (P_o) of the Cl^- channel in cell-attached membrane patches. The patch membranes are depolarized from the spontaneous resting potential for positive ΔV_m and vice versa. Lozenges and bars indicate mean values ±SEM from four patches

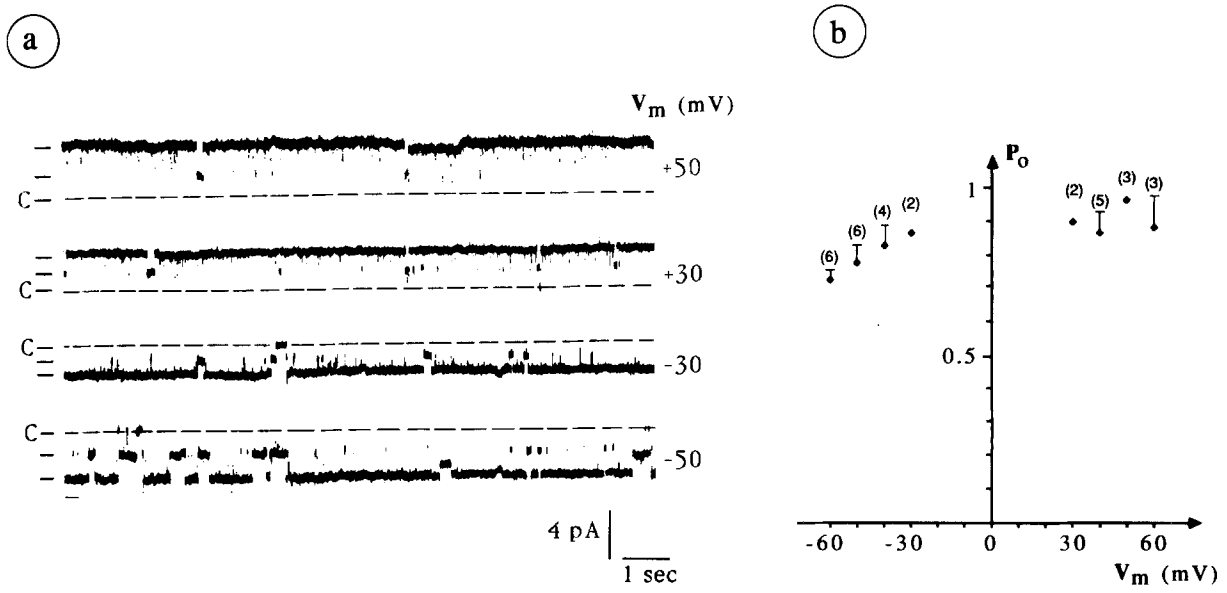

Fig. 3. Single-channel records and P_o-voltage relationship for excised, inside-out membrane patches. The patch pipette and the bath contained NaCl Ringer solution with Ca^{2+} concentrations of 10^{-9} and 10^{-7} M, respectively. The transmembrane potential (V_m) is expressed with the pipette interior as reference. (*a*) Representative single-channel traces for one inside-out patch are shown with the corresponding V_m next to each trace; outward currents are upwards. Records were filtered at 800 Hz low pass. (*b*) P_o-voltage relationship; lozenges and bars indicate mean values ±SEM from two to six patches as denoted by numbers in parentheses

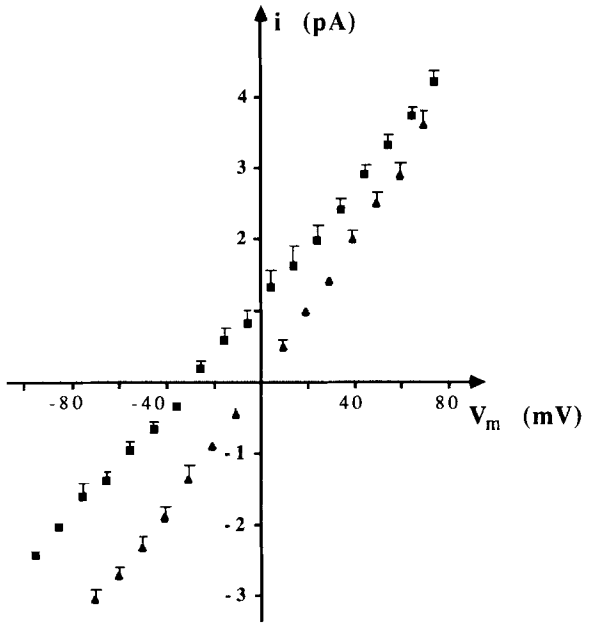

Fig. 4. Current-voltage relationships for Cl^- channels recorded from excised, inside-out, membrane patches with 140 mM NaCl-Ringer solutions bathing both sides of patch membranes (triangles) or with 140 mM NaCl-Ringer solution bathing the external and 42 mM NaCl solution bathing the internal surfaces of the patch membrane (squares). Plots are mean values from eight membrane patches and SEM when they are larger than symbols are indicated by vertical bars. *i*, single-channel current; V_m, membrane potential

Conductance and Selectivity Properties

Figure 3*a* shows membrane currents recorded from one inside-out membrane patch bathed in symmetrical NaCl-Ringer solutions. The free Ca^{2+} concentration in the bath was 10^{-7} M (unless otherwise stated). This calcium concentration is below the threshold for opening the Ca^{2+}-activated nonselective cation channel (Teulon et al., 1987). Under these conditions, the 40-pS channel was the only observed ionic channel, except for an occasional smaller 8–10 pS conductance. As previously determined (Paulais & Teulon, 1989), the latter is cation selective. In contrast to what was observed for cell-attached membrane patches, there was no clear transmembrane voltage dependence of channel activity in inside-out membrane patches; P_o was close to 0.8 at all voltages tested (between −60 and +60 mV membrane potential) (Fig. 3*b*).

The ionic selectivity of the channel was obtained from plots of the single-channel current amplitude against membrane voltage (*I-V*) under a variety of ionic gradients across the membrane patch. With symmetrical 140 mM NaCl conditions the *I-V* relationship was linear, with a mean unitary conductance of 44.8 ± 0.9 pS (n = 15) (Fig. 4). When the NaCl concentration in the bath solution was 42 mM and with 140 mM NaCl in the pipette, the reversal potential of the *I-V* relationship was shifted to

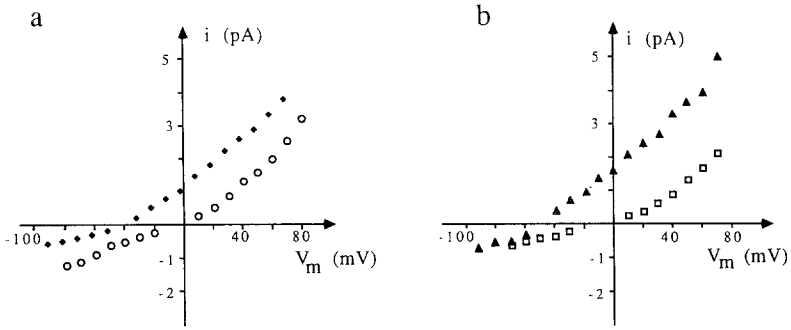

Fig. 5. Anion selectivity of cTALH Cl^- channel. (*a*) Current-voltage relationships obtained with 130 mM chloride in the bath replaced with bromide (n = 4, open circles) or fluoride (n = 4, lozenges). (*b*) Current-voltage relationships obtained with 130 mM chloride replaced with nitrate (n = 5, open squares) or gluconate (n = 5, triangles). Patch pipettes were filled with 140 mM NaCl-Ringer solution. *i*, single-channel current; V_m, membrane potential. SEM are indicated by vertical bars when they are larger than symbols

-28.2 ± 1 mV (n = 8), a value close to the -32 mV predicted for a perfectly anion-selective channel (Fig. 4). From this figure, we derived a relative permeability for Na^+ (as compared to Cl^-) of 0.05.

Selectivity among anions was determined from shifts in the reversal potential when most of the chloride in the bath was replaced by a test anion. With 140 mM NaCl in the pipette and 140 mM NaCl in the bath, the channel reversal potential was close to zero. The *I-V* relationships obtained with Br^- (Fig. 5*a*), F^- (Fig. 5*a*), NO_3^- (Fig. 5*b*) as the major anions in the bath (130 mM) indicate that Cl^- is the most permeant species. The corresponding reversal potentials are -7.1 ± 1.5 mV (n = 7) for Br^-, -17.6 ± 2.4 mV (n = 5) for NO_3^- and -45.3 ± 0.5 (n = 4) for F^-. All values are statistically different from the equilibrium potential for chloride under these experimental conditions, $E_{Cl} = -57.4$ mV, at the 0.01 level. They yield the following permeability sequence:

$$P_{Br^-}/P_{Cl^-}(0.7) > P_{NO_3^-}/P_{Cl^-}(0.4) > P_{F^-}/P_{Cl^-}(0.03).$$

When 130 mM gluconate was substituted for chloride in the bath, the *I-V* relationship reversed at -48.9 ± 0.8 mV (n = 4), a value not statistically different from E_{Cl} (Fig. 5*b*). The relative permeability for iodide could not be determined since this ion blocked the Cl^- channel activity. When the cytoplasmic face of an inside-out membrane patch was exposed to 140 mM NaI, no Cl^- channel activity could be detected for either positive or negative applied membrane potentials (n = 4). We have not further investigated this point at this stage.

Diphenylamine-2-carboxylate (DPC), a blocker of basolateral Cl^- conductance in the rabbit cTALH (Wangemann et al., 1986), appeared to reversibly decrease the unitary current amplitude of excised, inside-out, membrane patches at each voltage tested (Fig. 6). DPC (2.5 mM) reduced the single-channel amplitude by about half (n = 2).

The activity of the cTALH Cl^- channel was not a function of cytoplasmic calcium: changing the

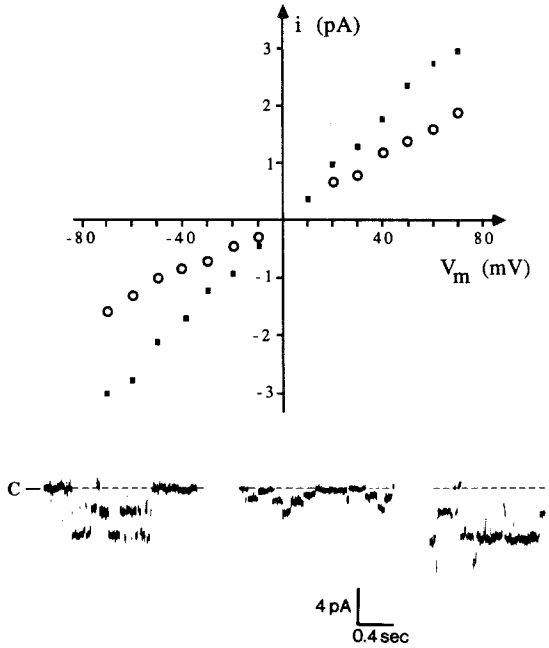

Fig. 6. The effect of diphenylamine-2-carboxylate (DPC) upon the current-voltage relationship of Cl^- channels recorded in symmetrical NaCl-Ringer solutions. The patches were first bathed in control Ringer solution (squares) then in Ringer solution containing 2.5 mM DPC (open circles). Beneath the graph are representative single-channel currents recorded at +30 mV membrane potential from the same membrane patch before (left), during (middle), and after (right), exposure to DPC

Ca^{2+} concentration ($[Ca^{2+}]$) in the bath from 10^{-7} to 10^{-9} (n = 3) or raising $[Ca^{2+}]$ to 10^{-3} M (n = 3) had no effect on the Cl^- channel activity (Fig. 7). However, long exposure (≈3–5 min) to high $[Ca^{2+}]$ (10^{-3} M) occasionally resulted in an irreversible decrease in channel activity (*not shown*).

Discussion

We describe for the first time the properties of an anion-selective channel of the basolateral mem-

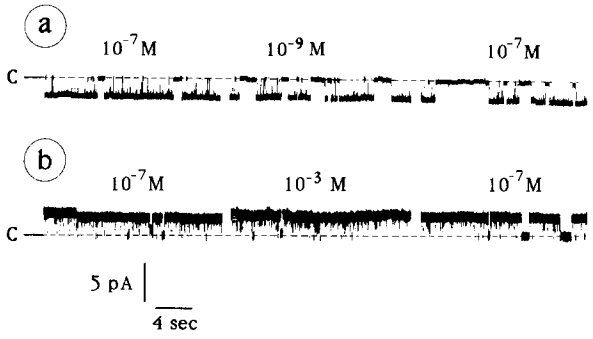

Fig. 7. Effect of internal calcium upon the Cl^- channel. (*a*) Single-channel recording from one inside-out membrane patch in symmetrical 140 mM NaCl-Ringer solutions; internal calcium was changed from 10^{-7} to 10^{-9} M; V_m = +50 mV membrane potential. (*b*) Single-channel recording from another inside-out membrane patch with 140 mM NaCl-Ringer solution bathing the external and a 42 mM NaCl solution bathing the internal surfaces of the patch membrane. The membrane potential was clamped at +30 mV, the reversal potential value for the Ca^{2+}-activated nonselective cation channel under these conditions

brane of the cTALH which might account for the basolateral Cl^- permeability of this part of the renal tubule (Greger & Schlatter, 1983). The channel has a linear *I-V* relationship with an unitary conductance of about 40 pS in symmetrical NaCl-Ringer solutions; its permeability to Na^+ is low and Cl^- is the most permeant anion.

The open-state probability is voltage dependent in cell-attached membrane patches, P_o increasing at positive voltages. Such enhancement of channel activity with membrane depolarization is typical of several Cl^- channels recorded from cell-attached (Frizzell et al., 1986*a*) or inside-out (Greger, Schlatter & Gögelein, 1987; Hayslett et al., 1987; Halm et al., 1988) membrane patches. However, no clear transmembrane voltage dependence has been found in excised, inside-out, membrane patches in our study. This could be because low P_o values observed at hyperpolarizing voltages for cell-attached membrane patches are shifted towards more negative voltage values in inside-out patches, outside the range of membrane potentials tested in these experiments (−60 to +60 mV). Such a negative shift of the P_o-voltage relationship in inside-out membrane patches as compared to cell-attached membrane patches has been reported for the outwardly rectifying Cl^- channel in a colonic tumor cell line (Halm et al., 1988).

With Cl^- being the most permeant species, the anionic equilibrium selectivity sequence of the cTALH channel ($Cl^- > Br^- > NO_3^- > F^-$) is not a very common one. Cl^- channels in the urinary bladder (Hanrahan, Alles & Lewis, 1985) and HT 29 human colonic carcinoma cells (Hayslett et al., 1987) discriminate poorly among anions. The Cl^- channels in other colonic cells (Reinhardt et al., 1987; Halm et al., 1988) and pulmonary alveolar cells (Schneider et al., 1985) have an equilibrium selectivity sequence analogous to Einsenmann's sequence 1 ($I^- > Br^- > Cl^- > F^-$) (Wright & Diamond, 1977). However, sequences analogous to that of the cTALH Cl^- channel have been found for an 11-pS Cl^- channel in the shark rectal gland ($Cl^- > Br^- \gg I^-$; Gögelein, Schlatter & Greger, 1987) and a 70-pS Cl^- channel from airway epithelial membranes reconstituted in artificial bilayers ($Cl^- > Br^- > F^- \gg I^-$; Valdivia, Dubinsky & Coronado, 1988). This corresponds to Einsenmann's sequence 4 or 5 (if we assume that permeability to iodide is not measurable); this suggests that the selectivity-determining site(s) within the channel has a relatively high charge density. We were surprised to find that the Cl^- channel was inhibited by the anion iodide. While we are not aware of a similar inhibition in other epithelial chloride channels, anion interactions with chloride conductances are known to occur in various muscle preparations, where reduction of Cl^- conductance by anions such as iodide, nitrate, cyanate have been reported (*see* Bretag, 1987).

Selective blockers of Cl^- channels are still under development, and the effect of a given substance may vary drastically from tissue to tissue (*see* for example Gögelein, 1988). Diphenylamine-2-carboxylate (DPC) blocks the Cl^- conductance of in vitro microperfused rabbit cTALH with a half-maximal inhibitory concentration of 2.6×10^{-5} M (Wangemann et al., 1986). Our results, obtained at the single-channel level in mouse cTALH, are at variance with the electrophysiological data on rabbit cTALH, since we found a half-maximal inhibitory concentration of about 2.5 mM for the Cl^- channel of mouse cTALH in inside-out membrane patches. Similar results have been reported by Welsh (1986*b*) for the outwardly rectifying Cl^- channel in primary cultured cells from canine airway epithelia (half-maximal inhibitory concentration: 1 mM). In contrast, 10^{-4} M DPC, or less, completely inhibited the Cl^- channel of the shark rectal gland when added to the inner face of the membrane (Greger et al., 1987). However, since a delay of 30–60 sec was typically observed before onset of inhibition, the authors suggest that DPC actually acts at the outer aspect of the channel protein, the delay being due to permeation of DPC through the patch membrane.

Cl^- channel currents were very seldom observed in cell-attached patches in the absence of a cellular cAMP-stimulating agent in the present study. By contrast, Cl^- currents were detected in 67% of inside-out membrane patches prepared from

cTALH fragments which had been exposed to a cAMP-stimulating agent for at least 10 min prior to patch clamping. This suggests that a cAMP-dependent process is necessary for Cl^- channel activation. Qualitatively similar results were reported by Welsh (1986*a*) for primary cultures of human tracheal epithelium where an outwardly rectifying Cl^- channel was not only observed in 28% of cell-attached membrane patches following exposition to isoproterenol, but was observed in a further 42% of membrane patches after excision from the cell. In view of the relatively wide temperature range that obtained during our study (20–30°C), we cannot exclude the effects of some thermal dependence upon the Cl^- channel activity, *in situ*. Such a temperature-dependent modulation has been demonstrated for the cAMP-mediated increase of a potassium current in guinea pig ventricular heart cells (Walsh, Begenisch & Kass, 1989). In our study, a similar modulation could possibly explain the lower proportion of patches with active Cl^- channels in the cell-attached mode. This point remains to be investigated. The cAMP-activated, outwardly rectifying Cl^- channel is the most intensively studied Cl^- channel in epithelia. It was first described in primary cultured cells from canine (Welsh, 1986*b*) and human (Frizzell et al., 1986*a*,*b*; Welsh, 1986*a*) airway epithelia but is also present in cultured HT29 (Hayslett et al., 1987) and T84 (Halm et al., 1988) colonic cells, lymphocytes (Chen et al., 1989) and fibroblasts (Bear, 1988). The regulation of this channel is disturbed in the trachea and lymphocytes of patients with cystic fibrosis (Schoumacher et al., 1987; Li et al., 1988; Chen et al., 1989). Activation by sustained membrane depolarization, rectification of current flow and an anionic-selectivity sequence analogous to Einsenmann's sequence 1 are characteristic properties which clearly distinguish this channel from the cTALH Cl^- channel. The cTALH Cl^- channel shares more properties with a Cl^- channel from airway epithelial membranes reconstituted in artificial lipid bilayers (Valdivia et al., 1988) and an anion-selective channel located at the apical membrane of the rectal gland tubules (Greger et al., 1987). These channels also have a linear *I-V* relationship with unitary conductances of 70 and 45 pS, respectively, and they appear to be regulated by cAMP-dependent processes. However these two channels differ from the cTALH Cl^- channel in other properties such as unitary conductance and P_o-voltage relationship for the former, anionic-selectivity sequence for the latter. It thus appears that the channel described in this study displays novel characteristics for a Cl^- conductance in an epithelium.

The role of hormones in regulation of overall NaCl absorption in the cTALH remains an open question. There is no doubt that the cTALH, with considerable variations among species, is the target of several hormones acting through the cAMP second messenger system (*see* Morel et al., 1981). The adenylate cyclase system of mouse cTALH is sensitive to PTH, ADH, calcitonin, glucagon, and β-adrenergics agonists (Morel et al., 1981; Wittner et al., 1988), but adenylate cyclase-mediated modulation of NaCl absorption has not been firmly established in this part of the thick ascending limb of Henle's loop. Several studies using in vitro single tubule microperfusion have shown that ADH had no effect on transepithelial voltage (Friedman & Andreoli, 1982; Wittner et al., 1988), resistance or Na^+ and Cl^- fluxes (Wittner et al., 1988). Friedman and Andreoli (1982) have shown that 10^{-3} dibutyryl-cAMP does not modify the transepithelial voltage across mouse cTALH. On the other hand, β-adrenergic agonists appear to enhance NaCl absorption in the whole thick ascending limb of Henle's loop from mouse kidney (Bailly et al., 1989). These contrasting results might indicate a multifaceted regulatory system, in agreement with our own results, which were obtained at room temperature and showed that cAMP-stimulating agents only opened a limited number of Cl^- channels *in situ*, but were nonetheless necessary for their activation. A different situation prevails in the medullary part of the thick ascending limb of Henle's loop. There, ADH (Friedman & Andreoli, 1982) β-adrenergic agonists (Bailly et al., 1989) and glucagon (de Rouffignac et al., 1989) have been shown to activate NaCl absorption. While there is still some controversy about the primary target of hormonal regulation, Schlatter and Greger (1985) have demonstrated that cAMP increases the basolateral Cl^- conductance in isolated perfused fragments of mouse medullary thick ascending limbs. In this context, further studies are required to compare the patterns of hormonal activation for this Cl^- channel in cortical and medullary parts of the mouse thick ascending limb of Henle's loop.

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