Biochimica et Biophysica Acta 905 (1987) 125–132
Elsevier

BBA 73788

A Ca^2-activated cation-selective channel in the basolateral membrane of the cortical thick ascending limb of Henle's loop of the mouse

J. Teulon, M. Paulais and M. Bouthier

INSERM U.192, Hôpital des Enfants-Malades, Paris (France)

(Received 26 June 1987)

Key words: Basolateral membrane; Patch clamp; Ion channel; Calcium; Henle's loop; (Mouse kidney)

The patch-clamp technique was used to investigate the properties of a cation-selective channel in the basolateral membrane of microdissected collagenase-treated fragments of cortical thick ascending limbs of Henle's loop from mouse kidney. The channel activity was seldom observed in cell-attached patches (2 out 15 studied cases). In inside-out excised patches immersed in symmetrical NaCl Ringer's solutions, the unit channel conductance was ohmic and ranged from 22 to 33 pS (mean, 26.8 ± 0.6 pS, $n = 24$). When NaCl was replaced by KCl ($n = 8$) or sodium gluconate ($n = 2$) on the cytoplasmic side of the membrane, single-channel currents still reversed at 0 mV and the conductance was unchanged. The reversal potential was +28.8 ± 0.4 mV ($n = 8$) when a NaCl concentration (140 vs. 42 mmol/l) gradient was applied, close to the expected value (approx. 30 mV) for a cation selective channel. The channel was found to discriminate poorly between Na^+, K^+, Cs^+, and Li^+ ions. The activity of the channel was not clearly voltage-dependent but was dependent upon the free Ca^{2+} concentration on the cytoplasmic side of the membrane. We conclude that the channel resembles the non-selective cation channel which has been previously described in several tissues.

Introduction

The number of publications on ionic channels in kidney cell membranes has increased dramatically in recent years [31]. However, most of the patch-clamp studies published to date have concentrated on the luminal membranes of isolated renal tubules or cultured kidney cells, and only a few have attempted to characterize the ionic channels in the basolateral membrane. Two main cation channels have been demonstrated in the luminal membrane of renal cells. First, Ca^{2+}- and voltage-activated K^+ channels with different unit conductances have been found in the luminal membranes of cortical collecting tubules (rabbit [18,19], rat [31]), proximal tubule (rabbit [10] amphibian [20]) and cultured renal cells [9,14,22]. Second, an amiloride-sensitive Na^+ channel has been found in the luminal membranes of rat cortical collecting tubules [32], rabbit proximal tubule [11] and A6 cultured cells [16].

In the basolateral membrane of renal cells, cation channels have been described only in the proximal tubule; they include a 40 pS K^+ channel in the rabbit [10] and a Ca^{2+}-independent K^+ channel activated by hyperpolarizing voltages in *Necturus* [20]. More recently, Gögelein and Greger [11] have described an ionic channel permeable to Na^+, K^+ and Cl^-.

The present report describes the properties of a cation-selective channel present in the basolateral

Abbreviations: cTAL, cortical thick ascending limbs of Henle's loop; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

Correspondence: J. Teulon, INSERM U.192, TT 6ET, 149, rue de Sèvres, 75743 Paris cedex 15, France.

0005-2736/87/$03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

membrane of the cortical think ascending limb of Henle's loop of the mouse kidney, an important site of salt absorption [13] and the target of several hormones [28]. While this channel is different from the kidney cell ionic channels described previously, it shares striking similarities with a non-selective cation channel present in several other tissues [3,24,25,37]. This study does not define the physiological significance of the cation channel for the cortical think ascending limb of Henle.

Methods

Preparation of tubules. Cortical thick ascending limbs of Henle (cTAL) were isolated from the kidneys of adult male mice by manual dissection after collagenase treatment. The enzyme treatment was designed both to facilitate the microdissection and allow access to the basolateral membrane by removing the basement membrane which covers the tubules. Enzymatic removal of the basement membrane is commonly used in basolateral patch-clamp studies of epithelia such as exocrine gland acini [6,8,25] and enterocytes [29,34]. The following procedure to prepare the tubules was adapted from Imbert et al. [21]: immediately after death, one kidney was thoroughly perfused with Na^+ Ringer's solution containing collagenase via retrograde perfusion of the renal vein [30]. Small pyramids of tissue were then sliced along the cortical-medullary axis of the kidney and incubated in collagenase-containing NaCl Ringer's solution for 30–60 min at a temperature between 30 and 37 °C. For each animal, the incubation time and temperature were varied within these limits to find the best combination for removal of the basement membrane. Two collagenase preparations were used (Worthington CLSPA and CLS II), both at a concentration of 200 U/ml.

Microdissection was performed under a stereomicroscope with the tissue immersed in ice-cold NaCl Ringer's solution (pH 7.4, 10^{-4} mol/l $CaCl_2$, 0.8 mmol/l NaH_2PO_4, 4 mmol/l Na_2HPO_4).

Patch-clamp recording conditions. A microdissected fragment of tubule was transferred into a chamber (vol. 1 ml) containing NaCl Ringer's solution, mounted on the stage of an Olympus CK inverted microscope and viewed at a magnification of 600 ×. Single-channel currents were recorded from excised inside-out patches of basolateral membranes of CTAL using the patch-clamp methods described by Hamill et al [15]. An LM-EPC 7 (List Electronics, Darmstadt, F.R.G.) patch-clamp amplifier was employed; the signal was recorded on FM magnetic tape (Euromag. 1, Enertec, Villacoublay, France) and simultaneously displayed on a storage oscilloscope (Tektronix, Beaserton, OR, U.S.A.). Signals were low-pass filtered (0.3-1.6 kHz) using a VBF/8 variable filter (Kemo Ltd., Beckenham, U.K.). Pipettes were made from microhematocrit capillary tubes (CHR Badram, Bizkerod, Denmark), pulled in two stages and coated with Sylgard (Dow Corning, Seneffe, Belgium) according to standard methods [15]. Pipettes filled with isotonic saline had a typical resistance of 3–20 MΩ. Seals were achieved by suction and seal resistance ranged between 3 and 20 GΩ (mean about 7 GΩ).

The reference electrode was a 0.5 mol/l KCl per 4% agar bridge connected to an Ag/AgCl half-cell. Consequently, liquid junction potentials occurred at the reference bridge when the composition of the bath solution was changed. These potentials were measured using a micropipette filled with 2.7 mol/l KCl and the recorded values (less than 3 mV except in the case of a chloride-to-gluconate change) were used to correct reversal potentials. An Ag/AgCl pellet alone was used as a reference electrode when no substitution for chloride was made.

The sign of the potential refers to the bath side with respect to the pipette interior. Accordingly, positive single-channel currents correspond to a cation flux into the pipette, i.e., outwards through the membrane.

The open probability of the channel was calculated in some instances. The analysis was carried out manually by playing back data through the Kemofilter into a high-speed ultraviolet recorder (OM4501, Schlumberger, Paris, France) equipped with a 1.8 kHz galvanometer at a speed of 10 cm/s. Openings of less than 0.5 ms were thus filtered out; we assumed that missing events had little effect on the determination of the total open times and on the subsequent estimation of the fraction of time during which a channel is open.

Media the standard NaCl Ringer's solution

contained (mmol/l): NaCl (140); KCl (4.5); $MgCl_2$ (1.1); Hepes (10); glucose (10). Test solutions were prepared by substitution of NaCl and KCl, other constituents remained unchanged. The high-K^+ solution contained 126 mmol/l KCl and 14 mmol/l NaCl; the low-Cl solution contained 140 mmol/l sodium gluconate and 4.5 mmol/l KCl; the NaCl-diluted solution contained 42 mmol/l NaCl, 200 mmol/l saccharose and no KCl. The cesium and lithium solutions contained 135 mmol/l CsCl or LiCl, 5 mmol/l NaCl and no KCl. Solutions used during the course of the experiment with excised patches were titrated to pH 7.2.

Ca^{2+} concentrations below 10^{-5} mol/l were determined by the addition of $CaCl_2$ and EGTA [7]. Ca-EGTa buffers were prepared by titration as described by Miller and Smith [27]. The free Ca^{2+} concentration was calculated using stability constants for all reactions between Ca^{2+}, H^+, Mg^+ and EGTA [4,5,23]. Constants involving a H^+ reaction were corrected, taking into account the fact that pH measurements result in values for activity rather than concentration (Ref. 4 according to Tsien).

A set of perfusion pipettes was used to test the effects of different Ca^{2+} concentrations on channel activity [37]. The perfusion rate was estimated

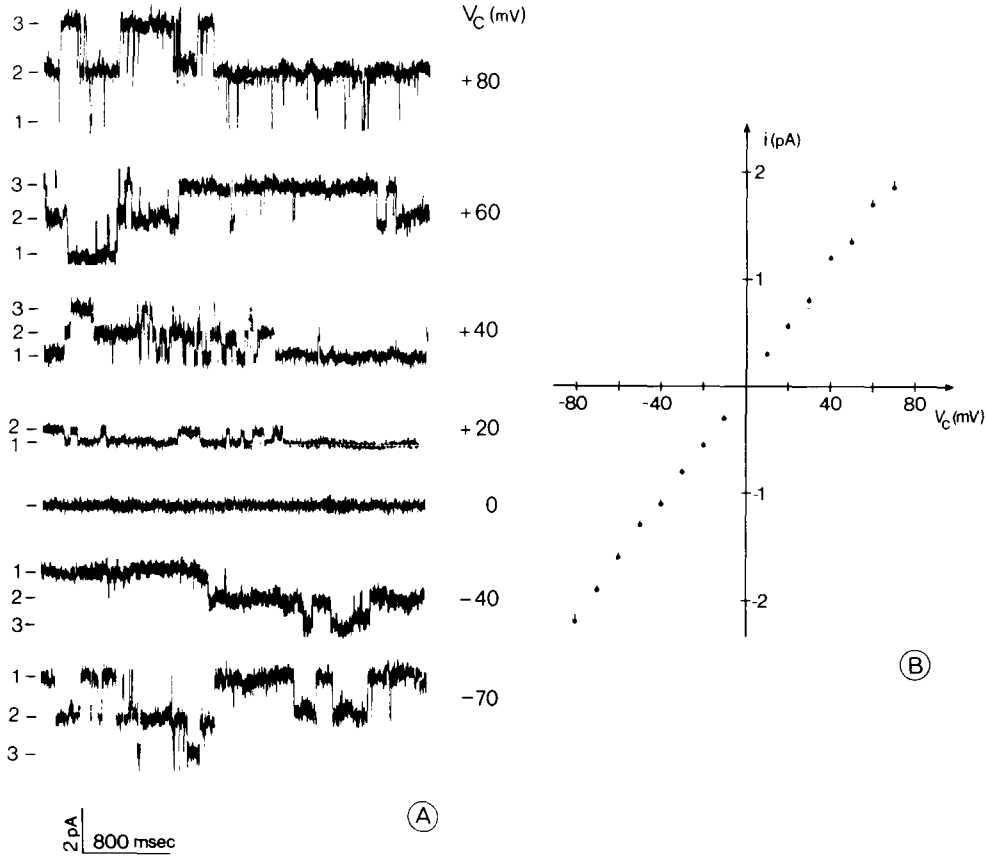

Fig. 1. (A) Unitary currents recorded from an excised inside-out patch in symmetrical NaCl Ringer's solutions (pH 7.2, $[Ca^{2+}]$ 10^{-4} mol/l). Single-channel current levels are indicated by relevant numbers, and level one was open throughout. V_c is the potential in the bath with respect to the pipette interior; outward membrane current is upward membrane current is upward; filtering, 300 Hz. (B) Single current channel (i) -voltage (V) relationship from excised inside-out patches in symmetrical NaCl Ringer's solutions. Plots are mean values from 24 membrane patches and S.E. are indicated by vertical bars when they are larger than the symbols.

to be 2–3 ml/h. Solution changes were performed by moving the patch pipette from the outflow of one pipette to the outflow of another pipette.

All experiments were carried out at room temperature (i.e., 23–30 °C).

Experimental values are given ± S.E., where n denotes the number of results.

Results

Typical membrane currents recorded from an inside-out patch, excised from basolateral membranes of cTAL fragments bathed in symmetrical NaCl Ringer's solutions are shown in Fig. 1a. We did not find any evidence for voltage-dependent activation of the channel ($n = 5$). Conductance through the channel was ohmic, as shown in Fig. 1b, and ranged from 22 to 33 pS (mean 26.8 ± 0.6 pS, $n = 24$).

Ion selectivity of this channel was studied in excised patches with NaCl Ringer's filled pipettes. Solutions were changed on the cytoplasmic side of the membrane (i.e., in the bath) from NaCl Ringer's to a test solution and I–V curves were plotted for both control and test conditions to determine shifts in reversal potentials. Substitution of high-K^+ solution produced no change in the reversal potential or in the unit conductance ($n = 8$, see Fig. 2a). This result could be due either to poor discrimination between sodium and potassium ions by a poorly selective cation channel or to chloride permeation through an anionic channel. When the bath solution was changed from a NaCl Ringer's to a NaCl-diluted solution (NaCl = 42 mmol/l), the reversal potential moved towards positive values: the I–V curve reverses at +28.8 ± 0.4 mV ($n = 8$, see Fig. 2b), close to the expected value for a cation selective channel (approx. 30 mV). Also, no change in the conductance nor in the reversal potential occurred when all but 10 mmol/l of chloride was replaced by gluconate ($n = 2$).

The alkali metal ions, lithium and cesium, permeated through the channel nearly as well as sodium and potassium ions. When most of sodium present in bath was replaced by lithium, the mean reversal potential for the I–V curve was 3.0 ±2.4 mV, ($n = 4$). Replacement of sodium by cesium gave a reversal potential of +7.5 ± 1.4 mV, ($n = 4$) (Fig. 3). From this value, a tentative P_{Cs}/P_{Na} of approx. 0.8 was calculated.

The rate of channel opening was dependent on

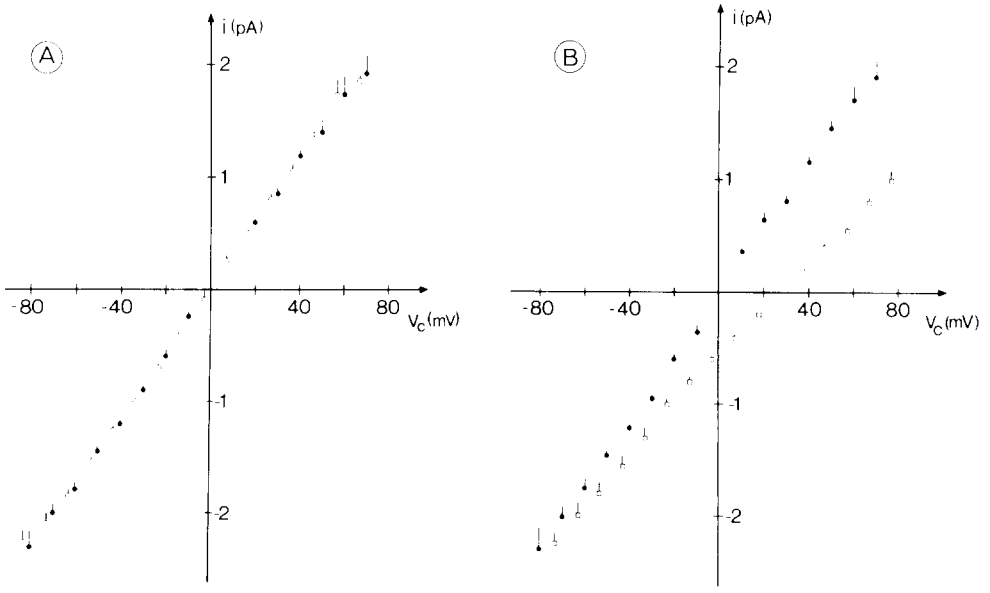

Fig. 2. Single-channel current (i) -voltage (V_c) relationships from excised patches for different bath solutions. (A) High-K^+ (126 mmol/l) Ringer's solution (open circles) and (B) NaCl-diluted (42 mmol/l) Ringer's solution (open squares). Filled circles represent i-V_c curves with a NaCl Ringer's solution in the bath (A,B); patch pipettes were filled with a NaCl Ringer's solution. Plots are mean values from eight membrane patches.

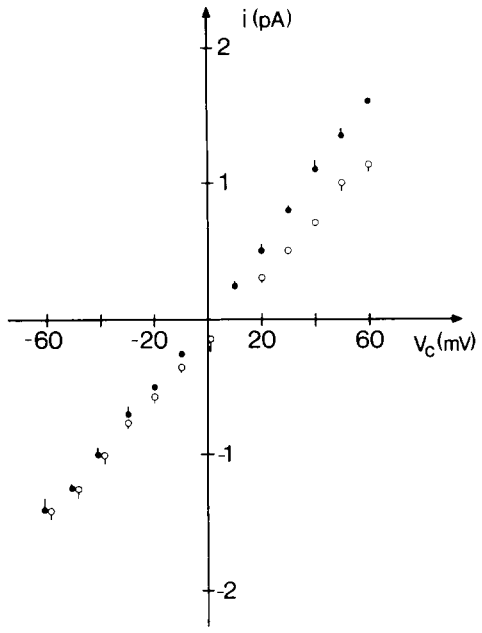

Fig. 3. Single-channel current (i) -voltage (V_c) relationships from excised patches for NaCl (filled circles) and CsCl (open circles) Ringer's solution. The pipette interior was filled with a NaCl Ringer's solution. Plots are mean values from four membrane patches.

the free Ca^{2+} concentration on the cytoplasmic side of the membrane. An example is given in Fig. 4: the patch pipette (filled with a NaCl Ringer's solution) was initially immersed in NaCl Ringer's solution containing 10^{-4} mol/l [Ca^{2+}]; lowering [Ca^{2+}] to 10^{-9} mol/l abolished channel activity; returning to 10^{-4} mol/l [Ca^{2+}] reactivated the patch and a return to 10^{-9} mol/l [Ca^{2+}] made it silent again. Fig. 5b summarizes the effects of free cytoplasmic [Ca^{2+}] on the open probability of the channel as observed in eight experiments. No channel activity was observed at [Ca^{2+}] of no greater than $5 \cdot 10^{-7}$ mol/l. Raising [Ca^{2+}] to higher values resulted in a definite increase of channel activity (see in Fig. 5a). However, the Ca^{2+} concentration necessary to activate the channel was quite high. Thus, we designed an experiment to verify whether a desensitization to calcium occurred with time in excised membrane patches. The perfusion chamber was rinsed with a 10^{-9} mol/l [Ca^{2+}] solution and subsequently equilibrated in a $5 \cdot 10^{-7}$ mol/l [Ca^{2+}] solution for several minutes. After a seal had been achieved, the pipette was withdrawn from the cell while

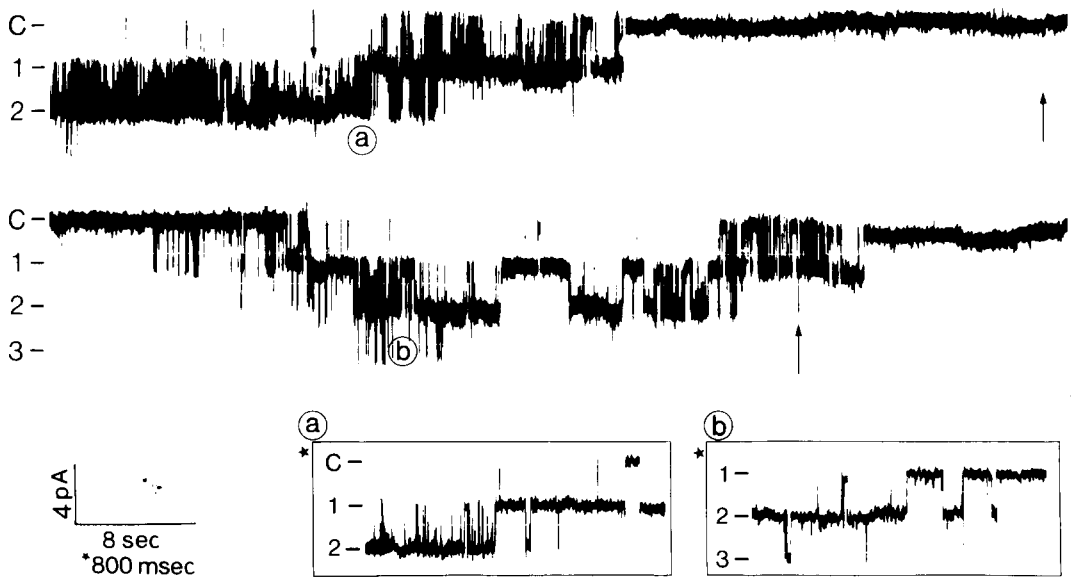

Fig. 4. Continuous recording from an excised inside-out patch showing the calcium dependence of the channel activity. The trace begins at 10^{-4} mol/l [Ca^{2+}]; the downward arrow indicates a solution change to one containing 10^{-9} mol/l [Ca^{2+}] and the upward arrow indicates a change back to 10^{-4} mol/l [Ca^{2+}]; both bath and pipette contain NaCl Ringer's solution at pH 7.2; [Ca^{2+}] in the pipette is 10^{-9} mol/l; $V_c = -80$ mV; filtering, 600 Hz. Insets a and b represent excerpts of the same recording on an expanded time scale. Three levels of single-channel currents are present in this record; they are indicated by relevant numbers; the letter C indicates the patch current when all channels were closed.

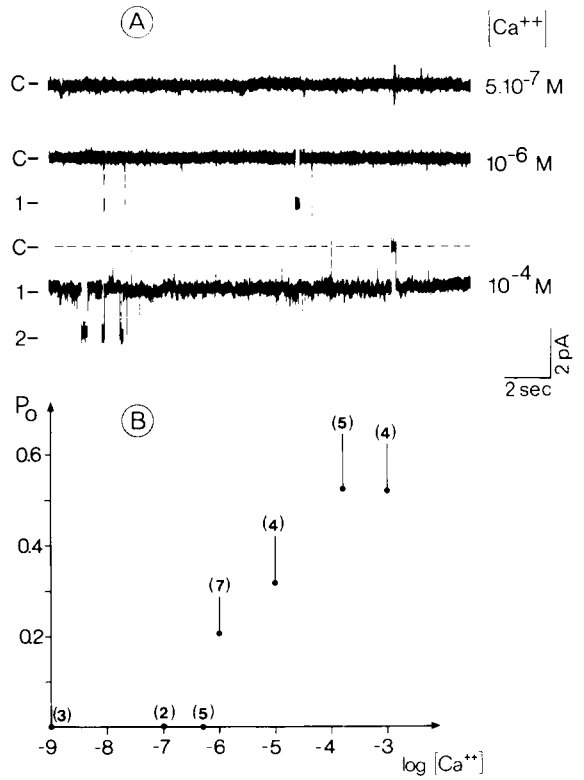

Fig. 5. Effects of free $[Ca^{2+}]$ present on the cytoplasmic side of the membrane on channel opening rate. (A) Recording from an excised inside-out patch showing the increase in channel activity when $[Ca^{2+}]$ increases from $5 \cdot 10^{-7}$ to $1 \cdot 10^{-4}$ mol/l perfusion pipettes contained a high-K^+ (126 mM) Ringer's solution and the patch pipette a NaCl Ringer's solution ($[Ca^{2+}] = 10^{-9}$ mol/l). $V_c = -60$ mV, filtering, 600 Hz. (B) Relationship between channel open probability (P_o) and 'cytoplasmic' free $[Ca^{2+}]$. Filled circles and bars indicate mean values ± S.E. from 2–7 patches as denoted by numbers in brackets. This figure summarizes the results from eight experiments.

maintaining voltage at −60 mV; clear unitary currents were observed after excision. A progressive decline of the activity took place and the channel eventually became fully inhibited in 2–3 min (two experiments). The closure was not irreversible and could be antagonized by increasing $[Ca^{2+}]$ to 10^{-6}, 10^{-5} and 10^{-3} mol/l.

The channel was seldom observed in cell-attached membrane patches (2 out of 15 experiments) under our experimental conditions (NaCl Ringer's solution in bath and pipette).

Discussion

The Ca^{2+}- and voltage-activated K^+ channel and the amiloride-sensitive Na^+ channel have been characterized in both renal cells [31] and in other secretory epithelia [35]. There are, however, several other types of cation channel in epithelia [35] which show different properties. This study demonstrates that the basolateral membrane of the mouse cTAL contains a cation channel which possesses the following characteristic features. (1) Although cation selective, it discriminates poorly between sodium and potassium ions. (2) Its permeability for anions is too small to be measured. (3) It has an ohmic type conductance of about 27 pS. (4) It is not clearly voltage dependent (5) The channel activity is dependent on cytoplasmic free $[Ca^{2+}]$.

The cTAL cation channel differs from the cation channels which have been described in renal cells [2,12]. In the apical membrane of the collecting duct principal cells in culture [2], a cation channel with a slightly larger permeability to potassium ions as compared to sodium ions has been described. Another channel, studied by Gögelein et al. [12], is present in the basolateral membrane of the late proximal tubule; it shares some properties of the cTAL cation channel. It has a comparable unit conductance of 28 pS, a linear current-voltage relationship and it does not discriminate between sodium and potassium ions. However, other properties are different; it is active in cell-attached patches, its activity is voltage-dependent and it has a measurable permeability for chloride.

While the cTAL cation channel differs from previously described cation channels in kidney cells, it resembles the non-selective cation channel characterized in several other tissues including mouse neuroblastoma cells [37], cultured rat heart muscle cells [3], basolateral membrane from mouse pancreatic acini [25,26] and rat lacrimal gland [24]. Similar properties were demonstrated for the channel in all the above tissues: unit channel conductances between 22 and 40 pS, linear current-voltage relationship, no voltage dependence, high selectivity for cations, no discrimination between sodium and potassium ions, channel activ-

ity dependent on free $[Ca^{2+}]$ present on the cytoplasmic side of the membrane.

The permeation of cations other than K^+ and Na^+ through the non-selective cation channel has only been studied in pancreatic acini [8] and neuroblastoma cells [37]. Rubidium [8] was found to permeate just as easily as sodium and potassium ions cesium and lithium [37] were slightly less permeant. The results obtained in this study when lithium or cesium were substituted for sodium are similar, and the cTAL cation channel also resembles the non-selective cation channel in this respect. Such poor cationic selectivity is not exceptional. Several ionic channels are similar to the non-selective cation channel in excluding anions but having a weak selective permeability to cations: the end-plate channel [1], a cation channel in the skin of the larval or adult frog [17,35], and a cation channel in the apical membrane of toad urinary bladder [36] are all permeable to a number of cations including Na^+, K^+, Cs^+, Li^+.

The effects of cytoplasmic Ca^{2+} on channel activation show that, as with the non-selective cation channel [3,24–26,37], the cTAL cation channel is inactive when $[Ca^{2+}]$ is lowered below 10^{-6} mol/l and the channel open probability increases with increasing $[Ca^{2+}]$ above a threshold value. Thus, the sensitivity to cytoplasmic Ca^{2+} appears to be low compared with that of Ca^{2+}- and voltage-activated K^+ channels; these channels are active at $[Ca^{2+}] = 10^{-7}$ mol/l [14,19] or even 10^{-8} mol/l [31], while the cTAL cation channel is completely closed at such $[Ca^{2+}]$ values. The basal cytoplasmic $[Ca^{2+}]$ in these cells, when measured with fluorescent dyes [30], is about 100 nmol/l, a value below the activation threshold of the cation channel recorded in this study. Thus, the channel should not be detectable in cell-attached patches, as was indeed observed in 13 out of 15 experiments. Although straightforward in this respect, the responsiveness of the cation channel to calcium is puzzling, because a large increase in internal $[Ca^{2+}]$ is necessary to activate the channel. Consequently, the channel is unlikely to play a physiological role in anything but extreme conditions. Though we cannot exclude the possibility that the cTAL channel remains inactive under most conditions, it could well be that Ca^{2+} is not the major determinant of channel activity in intact cells. Some other, unknown, agent may be able to activate the channel or the responsiveness to calcium could decrease with passage of time in excised patches. For example, Petersen and Maruyama [26,33] reported a marked desensitization of non-selective cation channels in excised inside-out membrane patches. Similarly, we have observed a transient activation of the channel, followed by complete closure when the membrane patch was excised from the cell in a $5 \cdot 10^{-7}$ mol/l $[Ca^{2+}]$ solution, and we were able to reactivate the channel by increasing calcium to higher values. Thus, it is likely that the cation channel described in this study loses some sensitivity to $[Ca^{2+}]$ over time in excised patches, as other non-selective cation channels do. It is, therefore, possible that the cTAL cation channel would be activated by Ca^{2+} levels lower than 10^{-6} mol/l in intact cells.

Acknowledgements

The technical assistance of M. Blonde and the secretarial work of F. Lagier are acknowledged. Y. Deris made the photographs. This study was supported by Centre National de la Recherche Scientifique GRECO 24.

References

1 Adams, D.J., Dwyer, T.M. and Hille, B. (1980) J. Gen. Physiol. 75, 493–510
2 Chad, C., Gitter, A., Beyenbach, K.W., Cross, P. and Frömter, E. (1986) (Abstract) Kidney Int. 23, 393
3 Colqhoun, D., Neher, E., Reuter, H. and Stevens, C.F. (1981) Nature 294, 752–754
4 Fabiato, A. (1981) J. Gen. Physiol. 78, 457–497
5 Fabiato, A. and Fabiato, F. (1979) J. Physiol. Paris 75, 463–505
6 Findlay, I. (1984) J. Physiol. 350, 179–195
7 Findlay, I., Dunne, M.J. and Petersen, O.H. (1985) J. Membr. Biol. 83, 169–175
8 Gallacher, D.V., Maruyama, Y. and Petersen, O.H. (1984) Pflügers Arch. 401, 361–367
9 Gitter, A.H., Beyenbach, K., Christie, W.C., Gross, P., Minuth, W.W. and Frömter, E. (1987) Pflügers, Arch. 408, 282–290
10 Gögelein, H. and Greger, R. (1984) Pflügers Arch. 401, 424–426
11 Gögelein, H. and Greger, R. (1986) Pflügers Arch. 406, 198–203
12 Gögelein, H. and Greger, R. (1986) Pflügers Arch. 407 (Suppl.), S142–S148
13 Greger, R. (1985) Physiol. Rev. 65, 760–792

14 Guggino, S.E., Suarez-Isla, B.A., Guggino, W.B., Green, N. and Sacktor, B. (1985) Am. J. Physiol. 249, F448–F455
15 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100
16 Hamilton, K.L. and Eaton, D.C. (1985) Am. J. Physiol. 249, C200–C207
17 Hillyard, S.D., Zeiske, W. and Van Driessche, W. (1982) Pflügers Arch. 394, 287–293
18 Hunter, M., Lopes, A.G., Boulpaep, E.L. and Giebisch, G.H. (1984) Proc. Natl. Acad. Sci. USA 81, 4237–4239
19 Hunter, M., Lopes, A.G., Boulpaep, E.L. and Giebisch, G.H. (1986) Am. J. Physiol. 251, F725–F733
20 Hunter, M. Kawahara, K. and Giebisch, G. (1986) Fed. Proc. 45, 2723–2726
21 Imbert, M., Chabardes, D., Montégut, M., Clique, A. and Morel, F. (1975) Pflügers Arch. 354, 213–228
22 Kolb, H.A., Brown, C.D. and Murer, H. (1986) J. Membr. Biol. 92, 207–215
23 Martell, A.E. and Smith, R.L. (1974) in Critical Stability Stability Constants. Vol. 1, Plenum Press, New York
24 Marty, A., Tan, Y.P. and Trautmann, A. (1984) J. Physiol. (London) 357, 293–325
25 Maruyama, Y. and Petersen, O.H. (1982) Nature 299, 159–161
26 Maruyama, Y. and Petersen, O.H. (1984) J. Membr. Biol. 81, 83–87
27 Miller, D.J. and Smith, G.L. (1984) Am. J. Physiol. 246, C160–C166
28 Morel, F. and Doucet, A. (1986) Physiol. Rev. 66, 377–446
29 Morris, A.P., Gallacher, D.V. and Lee, A.C. (1986) FEBS Lett. 206, 87–92
30 Murphy, E., Chamberlin, M.E. and Mandel, L.J. (1986) Am. J. Physiol. 251, C491–C495
31 Palmer, L.G. (1986) Am. J. Physiol. 250, F379–F385
32 Palmer, L.G. and Frindt, G. (1986) Proc. Natl. Acad. Sci. USA 83, 2767–2770
33 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696
34 Sepulveda, F.V. and Mason, W.T. (1985) FEBS Lett. 191, 87–91
35 Van Driessche, W. and Zeiske, W. (1985) Physiol. Rev. 65, 833–903
36 Van Driessche, W. Aclvoet, I. and Erlij, D. (1987) Proc. Natl. Acad. Sci. USA 84, 313–317
37 Yellen, G. (1982) Nature 296, 357–359