Biochimica et Biophysica Acta, 978 (1989) 134–144
Elsevier

BBA 74273

Two types of K^+ channels in the apical membrane of rabbit proximal tubule in primary culture

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(Received 13 June 1988)
(Revised manuscript received 15 September 1988)

Key words: Potassium ion channel; Proximal tubule; Apical membrane; Patch clamp; (Rabbit)

The patch-clamp technique was used to investigate ionic channels in the apical membrane of rabbit proximal tubule cells in primary culture. Cell-attached recordings revealed the presence of a highly selective K^+ channel with a conductance of 130 pS. The channel activity was increased with membrane depolarization. Experiments performed on excised patches showed that the channel activity depended on the free Ca^{2+} concentration on the cytoplasmic face of the membrane and that decreasing the cytoplasmic pH from 7.2 to 6.0 also decreased the channel activity. In symmetrical 140 mM KCl solutions the channel conductance was 200 pS. The channel was blocked by barium, tetraethylammonium and *Leiurus quinquestriatus* scorpion venom (from which charybdotoxin is extracted) when applied to the extracellular face of the channel. Barium and quinidine also blocked the channel when applied to the cytoplasmic face of the membrane. Another K^+ channel with a conductance of 42 pS in symmetrical KCl solutions was also observed in excised patches. The channel was blocked by barium and apamin, but not by tetraethylammonium applied to the extracellular face of the membrane. Using the whole-cell recording configuration we determined a K^+ conductance of 4.96 nS per cell that was blocked by 65% when 10 mM tetraethylammonium was applied to the bathing medium.

Introduction

The development of the patch-clamp methodology in renal physiology permits characterization of the ionic channels present in the membrane of the cells constituting the different nephron segments. Although the basolateral membrane is accessible to the patch pipette, access to the apical membrane, however, remains difficult along all the nephron segments. To overcome this problem in the cortical collecting tubule, several authors [1–4] opened a microdissected tubule to gain access to the apical membrane. Gogelein and Greger [5], however, chose to approach the apical membrane of proximal straight tubule cells by introducing the patch pipette into the free end of an in vitro perfused tubule. Although very elegant, in practice, these techniques are difficult, the size of the nephron segment, in particular, presents a serious limitation. Recently, cultured renal cell methodology has been used to investigate various aspects of epithelial biology. In order to study the ionic channels located in the apical membrane of proximal convoluted tubules, we undertook the culture of microdissected S1 segments. The simple geometry and the free access to the apical membrane of the cultured monolayers present an experimental tool well adapted to electrophysiological techniques. However, if one's interest is in the transport properties of a specific nephron segment, problems arise with the segmental heterogeneity of the nephron. For this reason, we used the microdissection method to be certain of the origin of the culture. In two previous papers [6,7], we presented evidence that microdissected proximal tubules (S1 segment) in primary culture retain the immunological, biochemical and electrophysiological characteristics of the original ephithelium. In the present paper, we describe two K^+ channels in the apical membrane of cultured proximal cells: a large conductance, Ca^{2+} and voltage-activated K^+ channel which shares many of the properties of the maxi K^+ channel described in many tissues [8–11] including renal epithelium [2,4] and a K^+ channel with a smaller conductance which is blocked by apamin.

Materials and Methods

Primary cultures

The primary cell culture technique was described in detail in a previous paper [6]. Briefly, the S1 portions of

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the proximal tubules were microdissected under sterile conditions from 4–5-week old female New Zealand rabbit kidneys. The kidneys were cut into small pyramids which were incubated in a dissection medium containing 0.1 mg/ml collagenase (Cooper, U.S.A.). The tubules were seeded in collagen-coated Petri dishes filled with a primary culture medium composed of an equal mixture of Dulbecco's modified Eagle medium (DMEM) and Ham F12 (Gibco) containing 15 mM $NaHCO_3$, 20 mM Hepes (pH 7.5), 2 mM glutamine, 5 μg/ml insulin, $5 \cdot 10^{-8}$ M dexamethasone, 10 ng/ml EGF, 5 μg/ml transferrin, $3 \cdot 10^{-8}$ M sodium selenite, $1 \cdot 10^{-8}$ M triiodothyronine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cultures were maintained at 37°C in a 5% CO_2 water-saturated atmosphere.

Enzyme studies

Enzyme analysis was performed on 12–15-day-old cultured proximal tubules. The enzyme assays were made at 37°C. Alkaline phosphatase was assayed with *p*-nitrophenyl phosphate as substrate at pH 8.6, as described by Hubscher and West [12]. Leucine aminopeptidase was determined by the technique described by Kramers and Robinson [13] using leucine *p*-nitroanilide as substrate. γ-Glutamyltransferase was measured using γ-glutamyl *p*-nitroanilide as substrate (Sigma list kit). Hexokinase was determined by Bergmeyer's method [14] on cultured cells isolated by trypsinization and permeabilized by a freeze-thawing procedure.

The results were normalized for the DNA content of each sample which was measured by a modification of the method of Switzer and Summer [15].

Cyclic AMP production in primary cultures

Cyclic adenosine monophosphate (cAMP) produced by 12–15-day-old cultured cells was measured by radioimmunoassay [15]. For these experiments, cultures were grown on permeable support [17]. The epithelia were incubated at 37°C for 5–15 min with 0.1 μg/ml parathyroid hormone (PTH 1–34) or 10^{-8} M arginine vasopressin (AVP) in the presence of 3-isobutyl-1-methylxanthine (IBMX). The hormones were added to both apical and basolateral sides of the culture well.

Patch clamp technique

The patch-clamp technique described by Hammil et al. [18] was applied to the apical membrane of 12–15-day-old primary cultures of proximal tubules grown on collagen-coated Petri dishes, since at this age the cells exhibited a clear morphological polarity [6,7]. Culture dishes were placed on the stage of an Olympus IMT2 microscope. Only cells of polygonal shape in the mosaic-like epithelium were investigated. Patch pipettes made from hematocrit capillaries were pulled in two steps with a vertical puller (Kopf, Tujunga, CA, U.S.A.) and coated with sylgard to reduced background noise. Pipettes had resistances of 2–5 megaohm in standard NaCl solution. Membrane currents were recorded with a patch-clamp amplifier LM EPC5 (List Electronic, Darmstadt F.R.G.). Experimental data were stored digitally on videocassette using a digital audioprocessor PCM 701 (Sony, Japan) coupled to a videocassette recorder (Sony, Japan). Whole-cell current analysis was performed on a storage oscilloscope (Enertec, Schlumberger). Single channel currents to be analyzed were transferred to a PC XT IBM computer digitized at 1–3 kHz after postfiltering at 0.5–1 kHz low pass with an 8 pole Bessel filter (Frequency Device, U.S.A.). The digitized recordings displayed on a graphic monitor were analyzed with a threshold level set at 50% of the channel amplitude. The opening probability was estimated by the ratio of open time of the channel to total time of recording. When more than one channel was present in the patch, the opening probability of level n, $P(n)$, was calculated with respect to level n-1. The open probability was expressed as the mean of $P(n)$ values. The Goldman-Hodgkin-Katz current equation

$$I = \frac{F^2}{RT} zPkV \frac{K_o - K_i e^{zFV/RT}}{1 - e^{zFV/RT}}$$

was used to describe the current-voltage relationship of the channel, R, T, F, Pk and z have their usual meaning. K_o and K_i are the K^+ activities on the extracellular and cystosolic side of the membrane.

Experiments were carried out at room temperature (20–22°C). The NaCl solution contained (in mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, pH 7.4 adjusted with NaOH. The KCl solution contained (in mM) 140 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes pH 7.2 adjusted with KOH. Ca^{2+} concentrations below 10^{-6} M were obtained with a Ca^{2+}/EGTA mixture.

The 35 mM KCl solution contained (in mM): 110 NaCl, 35 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, pH 7.4 adjusted with NaOH. Throughout this paper the sign of the potential refers to the pipette interior with respect to the bath.

Results

Primary cultures of proximal cells

In the present study only the S1 segment of the proximal tubule was microdissected. The individual segment was seeded on a collagen-coated Petri dish. After a latent period of 2–5 days, exponential growth took place and persisted for up to 30 days. For patch-clamp analysis, the cultures were used after 12–15 days of seeding. The biochemical, immunological and morphological characteristics of the cultured proximal tubules have been analyzed in detail in two previous papers [6,7]. However, a summary of the enzyme activities of 12–15-day-old primary cultures is reported in Table I.

TABLE I

Enzymatic characteristics of 12–15-day-old cultured proximal and connecting tubules

Leucine aminopeptidase, γ-glutamyltransferase, alkaline phosphatase and hexokinase activities were measured in 12–15-day-old primary cultures of proximal and connecting tubules. Activities are expressed in nmol/min per μg DNA. Values are means ± S.E. of *n* experiments.

	Cultures of proximal tubules	Cultures of connecting tubules
Leucine aminopeptidase	1.65 ± 0.20 ($n = 35$)	0.95 ± 0.14 ($n = 16$)
γ-Glutamyltransferase	3.07 ± 0.35 ($n = 51$)	0.34 ± 0.10 ($n = 15$)
Alkaline phosphatase	0.85 ± 0.09 ($n = 34$)	0.14 ± 0.04 ($n = 14$)
Hexokinase	0.47 ± 0.07 ($n = 18$)	1.01 ± 0.12 ($n = 11$)

This table also gives, for comparison, data from isolated connecting tubules in primary culture under exactly the same conditions. Cultured proximal tubules exhibited high activities of the brush-border enzymes γ-glutamyltransferase, alkaline phosphatase and leucine aminopeptidase, whereas a low activity level of glycolytic enzyme was found. As expected the membrane-associated enzyme activities were significantly higher in the proximal than in the distal cultures and hexokinase activity was greater in cultures of connecting tubules. Another method of characterizing the nature of the primary culture is using the effect of peptide hormones on cyclic AMP production. After 5–15 min exposure to parathyroid hormone (PTH), cAMP increased 7-fold in the proximal cell layer cultures (Table II), but only 2.5-fold in the cultures of connecting tubules. Arginine vasopressin (AVP), on the other hand, did not significantly modify cAMP production in proximal cultures, but tripled it in distal cultures (Table II).

TABLE II

Adenylate cyclase activity in cultured proximal and connecting tubules

Cyclic AMP production in fmol/5 min per μg of DNA was measured in 12–15-day-old primary cultures of proximal and connecting tubules. Arginine vasopressin (AVP) and parathyroid hormone (PTH) concentrations were 10^{-8} M and 0.1 μg/ml, respectively. Values are means ± S.E. of *n* experiments.

Cultures	Control	AVP	PTH
Proximal tubules	534.8 ± 184.2 ($n = 6$)	404.2 ± 67.1 ($n = 3$)	3695.9 ± 1193.5 ** ($n = 6$)
Connecting tubules	179.5 ± 15.6 ($n = 8$)	545.1 ± 203.2 * ($n = 7$)	422.7 ± 110.9 ** ($n = 4$)

* $P < 0.1$; ** $P < 0.02$: statistical significance between stimulated and basal productions of cAMP.

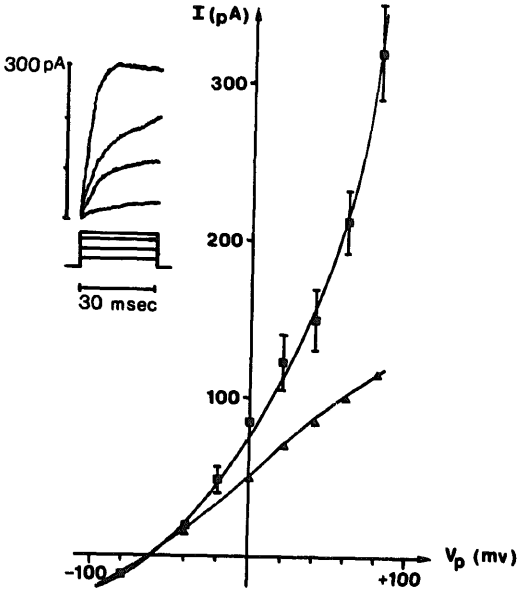


Fig. 1. Current-voltage relationship obtained in the whole-cell recording configuration. The pipette contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes (pH 7.2). The calcium concentration was 10^{-7} M (buffered with EGTA). The bath contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.4) (■) or in addition 10 mM tetraethylammonium (▲). The inset gives examples of current recordings obtained at three depolarizing potentials (−20, +20, +60, +80 mV) from the holding potential of −60 mV. V_p is the pipette holding potential with respect to the bath.

Whole-cell analysis

Applying a slight suction to the patch pipette resulted in the easy formation of a gigaseal (> 10 gigaohm) with the apical membrane, in more than 85% of trials. Whole-cell current recordings could be performed for 10–15 min. The inset of Fig. 1 shows current recordings obtained from an initial holding potential of −60 mV followed by depolarizing voltage steps. The *I/V* relationship is the mean of four recordings obtained in different cultures. When a series of electrical pulses from −80 to +80 mV was passed, the current was directed from the cell interior to the bath only at depolarizing voltages. In two experiments we tested the effect of 10 mM tetraethylammonium applied externally. Fig. 1 shows that about 65% of the current was blocked by the inhibitor. These data indicate that the recorded currents were mainly due to K^+ diffusion through K^+ channels. The K^+ conductance calculated from the maximal slope of the *I/V* curve was 4.96 ± 1.4 nS per cell.

Maxi K^+ channels

Cell-attached experiments

In order to characterize the ionic channels present in the apical membrane of proximal cells in primary culture, single-channel currents were recorded in the cell-attached configuration. A recording of the channel ac-

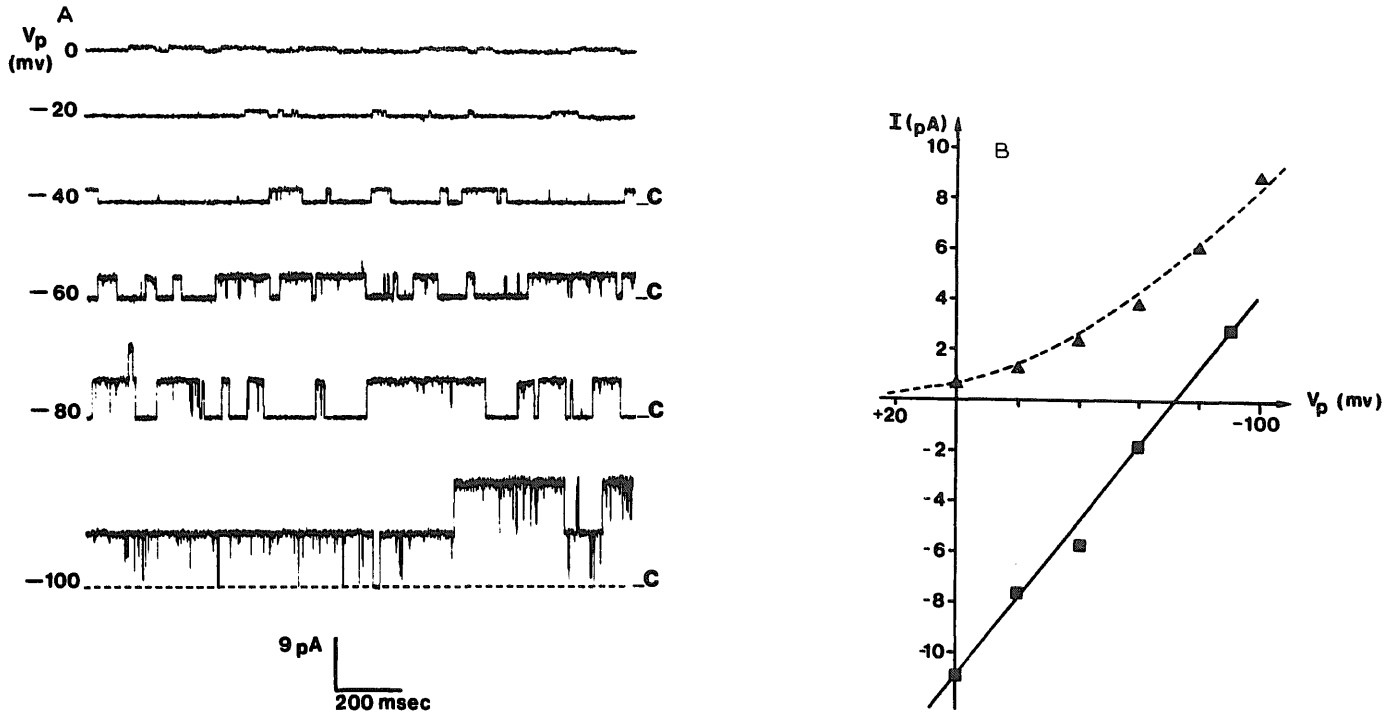


Fig. 2. (A) Single-channel current recordings in a cell-attached patch containing two active channels. Pipette and bath contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.4). Channel openings are indicated by upward deflections. C indicates the closed state of the channels. V_p is the pipette holding potential with respect to the bath. (B) Current-voltage relationship of the channel in the cell-attached configuration. The bath contained the same medium as described in A. The pipette contained the same solution (▲) or in (mM): 140 KCl, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.2) (■). The dotted line represents the Goldman-Hodgkin-Katz relationship. An intracellular K^+ concentration of 140 mM and a cellular membrane potential of −60 mV was assumed. V_p is the pipette potential with respect to the bath.

tivity most commonly observed is given in Fig. 2A. Under control conditions, with NaCl solution in the pipette and in the bath, an upward deflection represents current carried by positive charges moving from the cell interior to the pipette. At the resting membrane potential ($V_p = 0$ mV) the channel was spontaneously active and the current was upward. The current amplitude and the channel activity increased when depolarizing potentials were applied to the patch membrane. Furthermore, from −80 mV there was evidence of the opening of a second channel. The mean current-voltage relationship of six experiments is shown in Fig. 2B. The relationship was not linear over the range of 0–100 mV pipette voltage. The constant field equation of Goldman Hodgkin and Katz was applied to the data, assuming that the channel was K^+-selective. As shown in Fig. 2B, the experimental data are described perfectly by the theoretical curve. Single-channel conductance ranged from 40 pS near the resting membrane potential ($V_p = 0$ mV) to 130 pS when the membrane patch was depolarized to 0 mV. The high K^+ selectivity of the channel was confirmed in two experiments in which the pipette was filled with KCl solution. Under these conditions, the current-voltage relationship became linear (Fig. 2B). At the resting membrane potential, the current flowed from the pipette into the cell and reversed at −74 mV, which corresponded to the potential needed to cancel the intracellular potential. The maximal conductance represented by the slope of the straight line was 157 pS.

The channel kinetics were clearly voltage-dependent. As illustrated in Fig. 3, the opening probability was an exponential function of the applied potential.

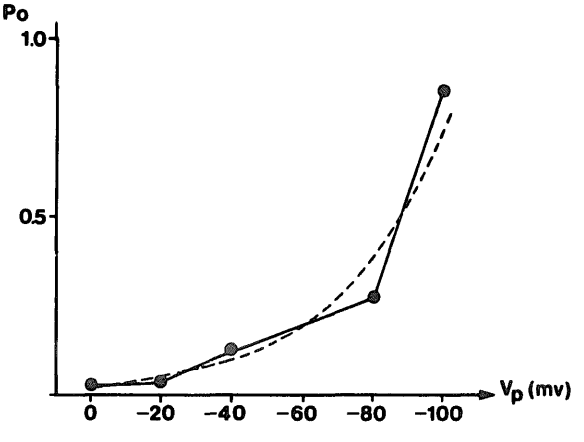


Fig. 3. Channel open probability versus pipette applied potential in the cell-attached configuration. The dotted line represents the fit according to an exponential function. V_p is the pipette holding potential with respect to the bath.

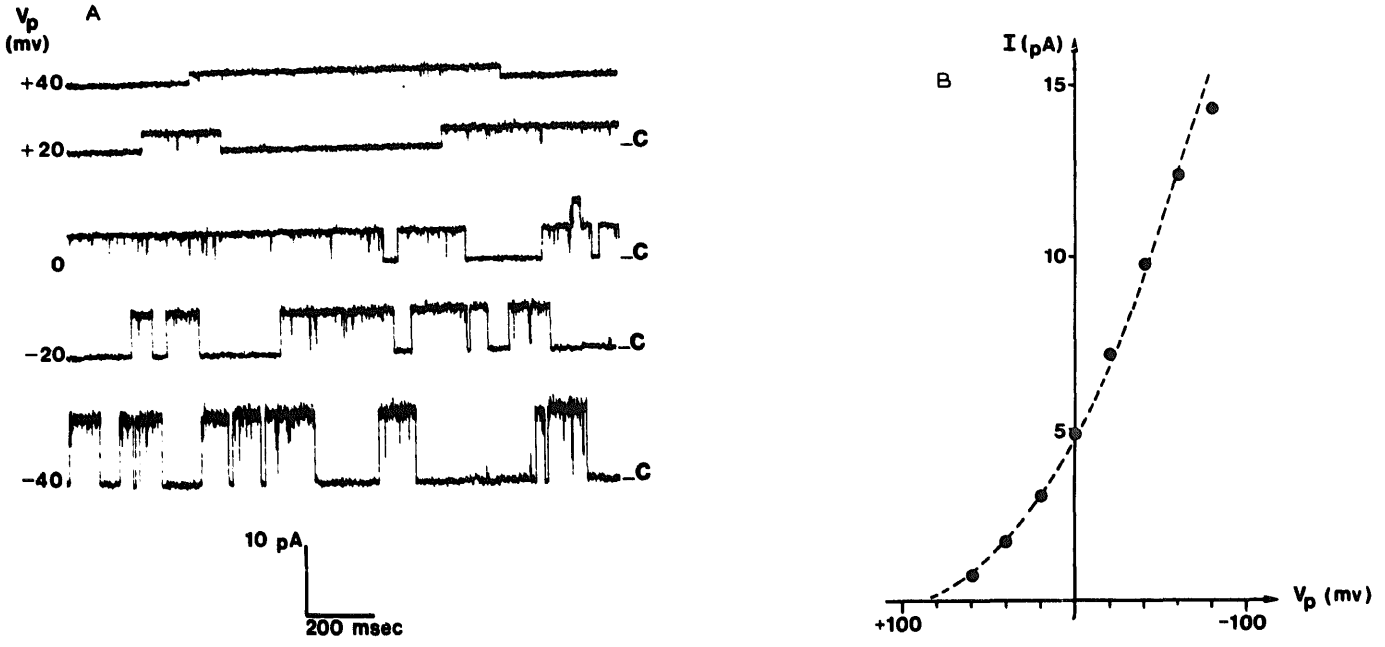


Fig. 4. (A) Channel current recording in an excised inside-out patch. The pipette contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 10 Hepes (pH 7.4). The bath contained (in mM): 140 KCl, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.2). C indicates the closed state of the channels. V_p is the pipette potential with respect to the bath. (B) Current-voltage relationship of the channel in excised inside-out patches. The pipette and bath contained the same media is for the experiments in A. The dotted line represents the Goldman-Hodgkin-Katz equation. V_p is the pipette holding potential with respect to the bath.

Excised patches

To investigate the characteristics of the channel further experiments were performed on excised patches.

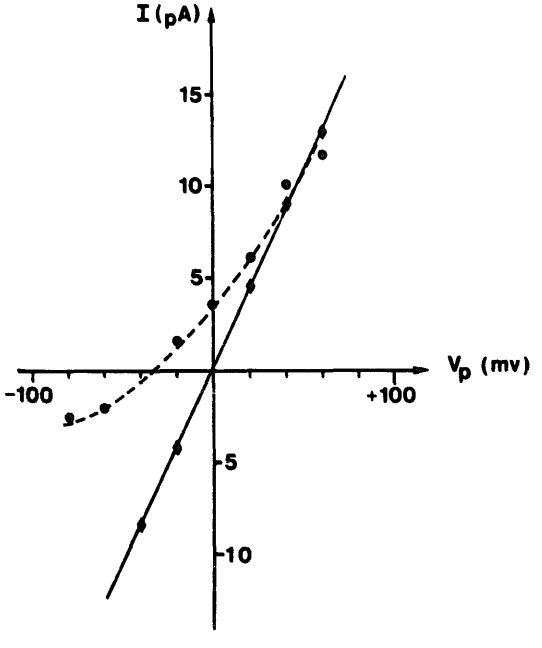


Fig. 5. Current-voltage relationship of the channel in excised right-side-out patches. The pipette contained (in mM): 140 KCl, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.2). The bath contained (in mM): 110 NaCl, 35 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.4) (●) or (in mM): 140 KCl, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.4) (◆). The dotted line represents the Goldman-Hodgkin-Katz equation. V_p is the pipette holding potential with respect to the bath.

Fig. 4A shows a recording of single-channel activity in an inside-out patch. The pipette contained NaCl solution and the bathing medium was the KCl solution. The channel current was always upward and its amplitude increased as the voltage applied passed from +40 to −40 mV. The current-voltage relationship of this channel is shown in Fig. 4B and agrees with the Goldman equation. As in the cell-attached experiments, we calculated a channel conductance ranging from 50 pS to 120 pS. In order to modify the K^+ concentration in the solution bathing the extracellular side of the patch membrane, rightside-out patches were obtained from a whole-cell configuration. With 140 KCl in the pipette and 35 KCl in the bath, the reversal potential shifted to −35 mV ($n = 2$). In this case, also the I/V curve (Fig. 5) was well described by the Goldman equation. The two conductances were 48 pS and 146 pS, respectively. When KCl solution was present on both sides of the membrane, the current reversal potential was 0 mV and the slope of the straight line gave a maximal conductance of 200 pS. The agreement between the data obtained in cell-attached and excised patches clearly indicates that the two types of experiment described the same channel.

Dependence on free Ca^{2+} concentration

To investigate whether this channel is Ca^{2+} activated, we examined the effect of the free Ca^{2+} concentration variation on the cytosolic face of an inside-out patch. The pipette was filled with 140 KCl, 10^{-6} M Ca^{2+} and

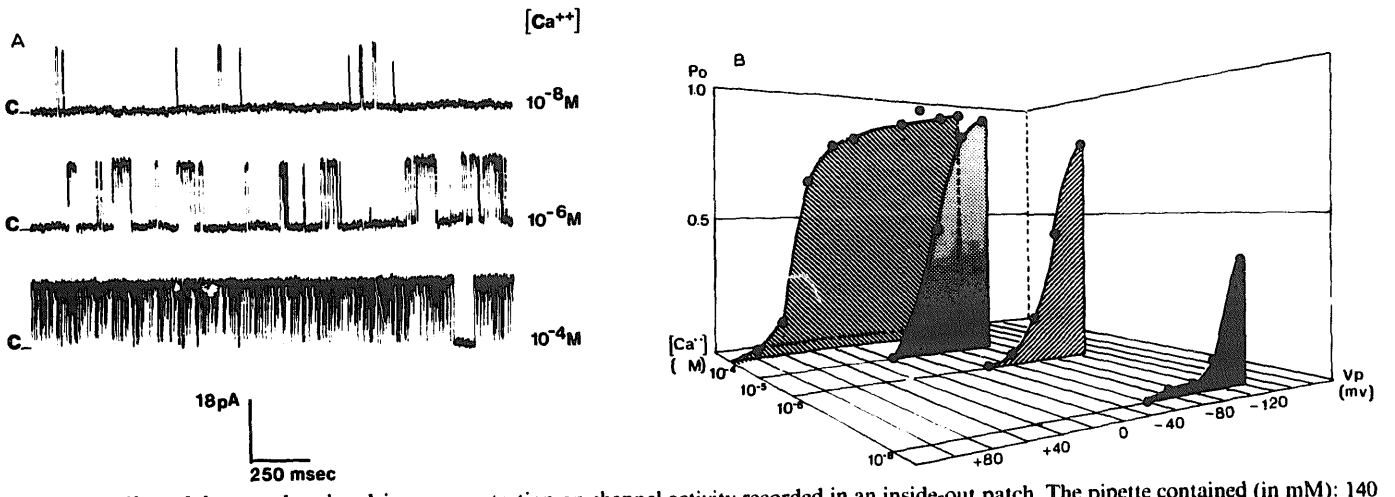


Fig. 6. (A) Effect of the cytoplasmic calcium concentration on channel activity recorded in an inside-out patch. The pipette contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes (pH 7.2). Calcium concentration was 10^{-6} M buffered with EGTA. The bath contained the same solution with different calcium concentrations ranging from 10^{-8} to 10^{-4} M. C indicates the closed state of the channel. The pipette holding potential was −80 mV with respect to the bath. (B) Three-dimensional plot of the channel-opening probability versus pipette applied potential at different cytoplasmic calcium concentrations. V_p is the pipette holding potential with respect to the bath.

the bath contained KCl solution with the free Ca^{2+} concentration ranging from 10^{-8} to 10^{-4} M. The holding potential was −80 mV. Fig. 6A gives an example of the current traces illustrating the increase of channel activity with increasing Ca^{2+} concentration. Fig. 6B shows the variation of channel-opening probability with the membrane potential at different Ca^{2+} concentrations.

Dependence on pH

To examine the pH dependence of K^+ channel activity, we varied the pH of the solutions at the cytoplasmic face of inside-out patches. The pipette was filled with KCl solution, 10^{-5} M Ca^{2+} and the bath contained the same solution but with two different pH values adjusted with 1 M HCl. Fig. 7A shows that the time during which the channel was closed was greater at pH 6.0. This is confirmed by plotting the channel-opening probability as a function of the membrane potential (Fig. 7B). This effect of pH was observed on three occasions.

Pharmacological properties

The influence of barium on K^+ channel activity was tested on the extracellular face of four rightside-ou

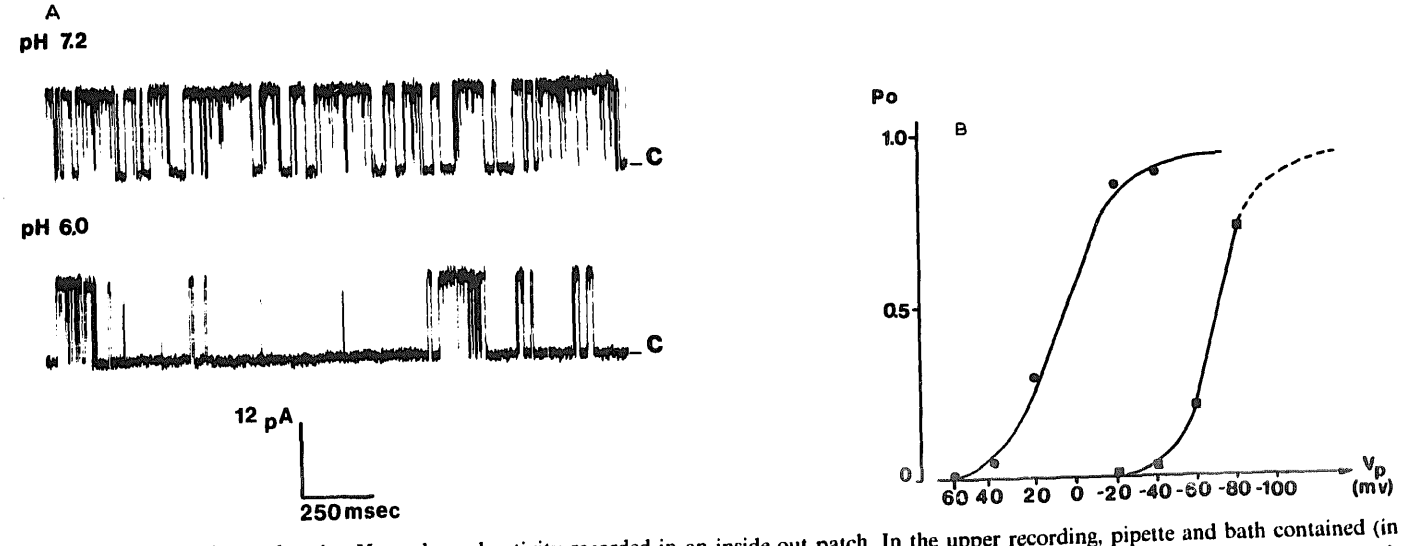


Fig. 7. (A) Effect of cytoplasmic pH on channel activity recorded in an inside-out patch. In the upper recording, pipette and bath contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 0.01 $CaCl_2$, 10 Hepes (pH 7.2). In the lower recording, the pH of the bathing solution was adjusted to 6.0 with HCl. C indicates the closed state of the channel. The pipette holding potential was −60 mV with respect to the bath. (B) Plot of channel-opening probability versus pipette applied potential with different cytoplasmic pH. Pipette and bath contained the same medium as described in A (●) or the same but at pH 6.0 (■) adjusted with HCl. V_p is the pipette applied potential with respect to the bath.

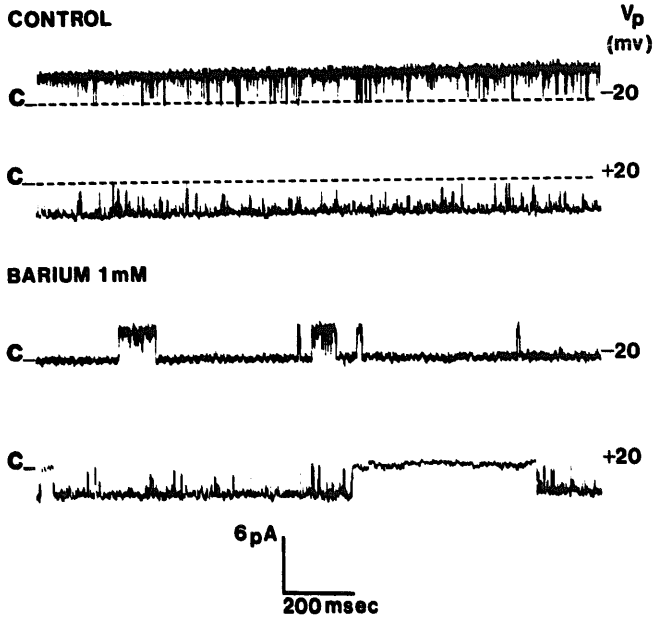


Fig. 8. Effect of 1 mM barium applied on the external face of an excised rightside-out patch at two pipette holding potentials (+20 and −20 mV). In control recordings, the pipette and the bath contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 10 Hepes (pH 7.2). In the bottom recordings, 1 mM barium was added to the bathing solution. The dotted line represents the baseline current level. C indicates the closed state of the channel. V_p is the pipette holding potential with respect to the bath.

patches. The results are illustrated in Fig. 8. The addition of 1 mM barium strongly decreased channel activity. The kinetics of the inhibition were characteristic of the action of slow blocking agents. Thus, Ba^{2+} reduced the length of the burst duration and induced long periods of inactivity between two bursts. The current amplitude was not modified. Fig. 8 clearly shows the voltage dependence of the Ba^{2+} blockage. Fig. 9 summarizes the

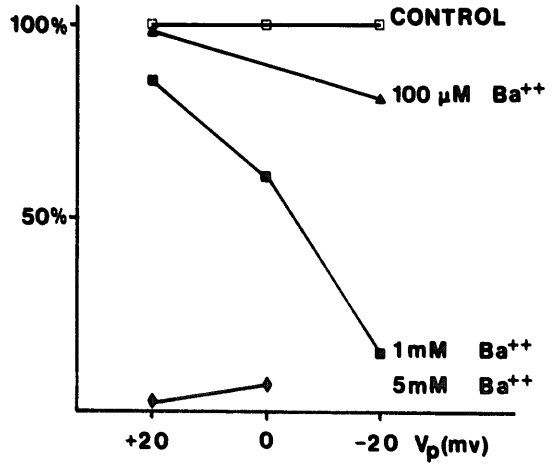


Fig. 9. Effect of voltage on barium blockage of the channel when barium was applied to the external face of excised rightside-out patches. Results are expressed in percent of channel-opening probability in control experiments (no barium added). V_p is the pipette holding potential with respect to the bath.

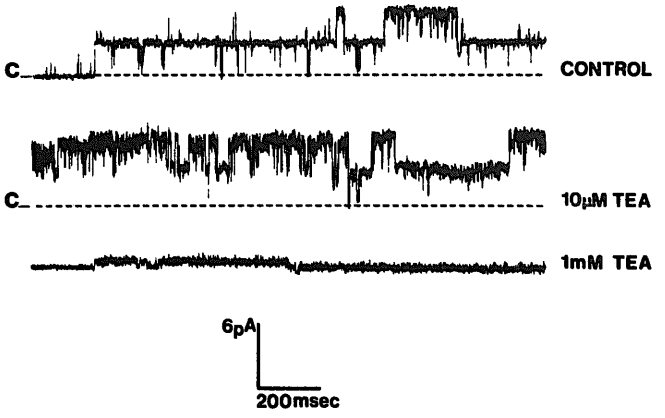


Fig. 10. Effect of external tetraethylammonium (TEA) on single-channel currents recorded in an excised rightside-out patch. In the control recording, the pipette contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 10 Hepes (pH 7.2) and the bath contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.4). In the lower recordings, 10 μM or 1 mM tetraethylammonium was added to the bathing solution. C indicates the closed state of the channel. The pipette holding potential was −30 mV with respect to bath.

data in terms of percent inhibition of channel activity as a function of voltage. At a high Ba^{2+} concentration (5 mM), the channel was completely blocked, irrespective of the potential applied to the patch membrane. At lower concentrations, the efficiency of the Ba^{2+} blockage increased with its electrochemical driving force. The influence of Ba^{2+} on the cytoplasmic face of the membrane was also tested in inside-out patches and similar conclusions were reached.

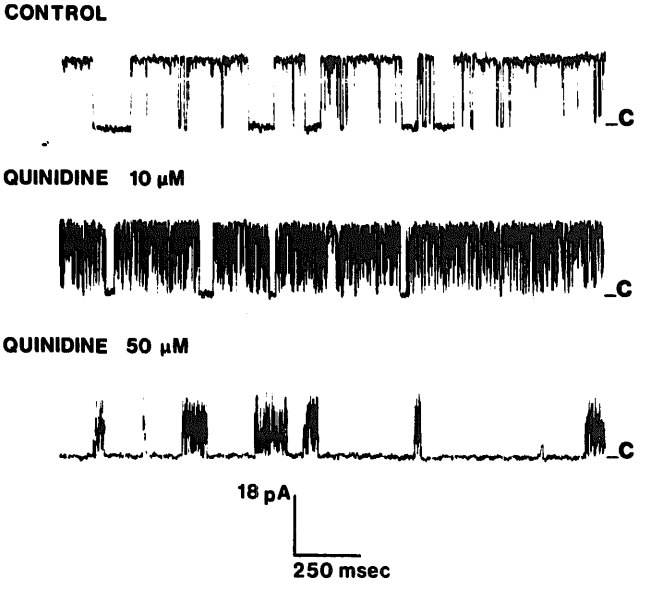


Fig. 11. Effect of quinidine applied on the cytoplasmic face of an excised inside-out patch. In the control recording, the pipette and bath contained (in mM): 140 KCl, 5 NaCl, 0.1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.2). In the lower recordings, 10 or 50 μM quinidine was added to the bathing solution. C indicates the closed state of the channel. The pipette holding potential was −80 mV with respect to the bath.

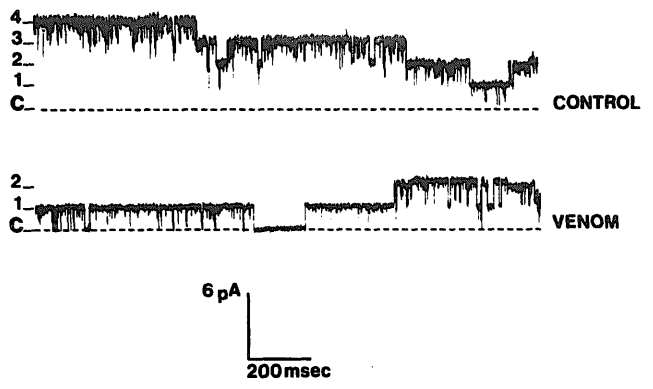


Fig. 12. Effect of crude scorpion venom (*Leiurus quinquestriatus*) on the extracellular face of an excised rightside-out patch containing four active channels. In the control recording, the pipette contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 10 Hepes (pH 7.2) and the bath contained (in mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.4). In the lower recording, scorpion venom was added to the bathing solution. Single-channel current levels are indicated by relevant numbers. C indicates the closed state of the channels. The pipette holding potential was −40 mV with respect to the bath.

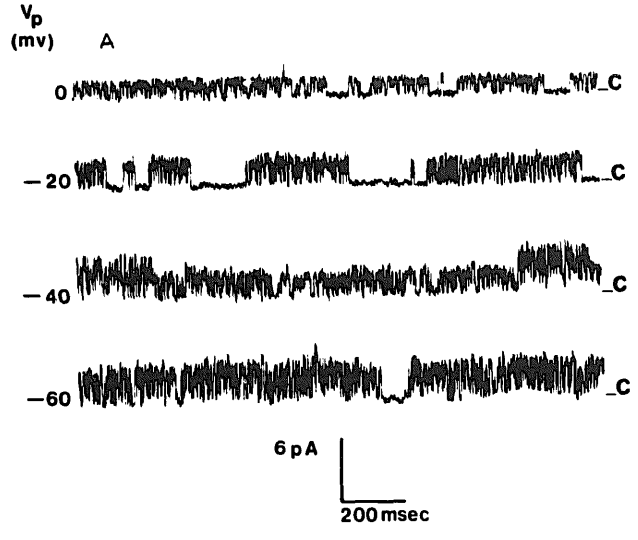
The effect of tetraethylammonium on the Ca^{2+}-activated K^+ channel was studied when the blocker was applied to the extracellular face of four rightside-out patches. One example of a recording obtained with 1 mM and 10 μM tetraethylammonium is given in Fig. 10. Tetraethylammonium at 1 mM decreased the current amplitude from 3.4 to 0.70 pA and reduced the opening probability from 0.44 to 0.15. At a low tetraethylammonium concentration (10 μM), the current amplitude was slightly reduced (by 20%) and the channel recording had a flickering appearance.

Quinidine applied to the internal face of three inside-out patches induced modifications of the K^+ channel activity similar to those obtained with extracellular tetraethylammonium. Fig. 11 shows a recording at −80 mV with 10 and 50 μM quinidine. Both effects of tetraethylammonium and quinidine were reversible when the drugs were rinsed out.

Fig. 12 illustrates the effect of crude *Leiurus quinquestriatus* venom on the Ca^{2+}-activated channel recorded in a rightside-out patch. Four channels were activated simultaneously in control conditions at −40 mV. Addition of venom to the external solution (final protein concentration: 12.5 μg/ml buffer solution) caused a significant reduction of the channel-opening probability from 0.47 ± 0.03 to 0.30 ± 0.03 ($n = 3$), without any apparent reduction of current amplitude.

Small K^+ channel

In excised patch experiments, we also recorded a second type of ionic channel. Fig. 13A is an example of the channel current obtained from an inside-out patch with KCl solution in the bath and NaCl solution in the pipette. At 0 mV, the current was upward and must



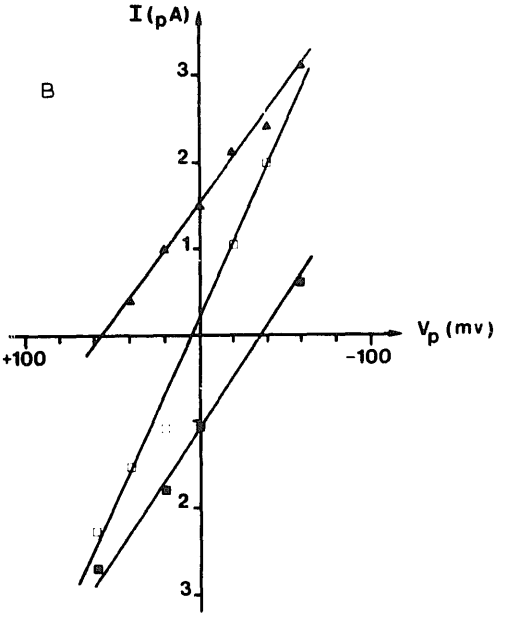


Fig. 13. (A) Single-channel current recordings of the small K^+ channel obtained in an excised inside-out patch. The pipette was filled with (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.4) and the bath contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.2). Channel openings are indicated by upward deflections. C indicates the closed state of the channel. V_p is the pipette holding potential with respect to bath. (B) Current-voltage relationship of the small K^+ channel. (▲) Corresponds to experiments performed on inside-out patches. The pipette contained the same medium as for A but at pH 7.2 and the bath contained the same media as for A. The two other straight lines correspond to experiments performed on rightside-out patches in which the pipette contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes (pH 7.2) (10^{-7} M Ca^{2+} buffered with EGTA) and the bath (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.2) (□) or 110 NaCl, 35 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.4) (■). V_p is the pipette holding potential with respect to the bath.

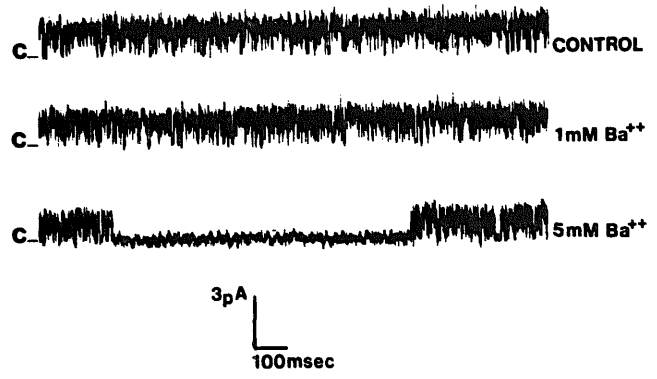


Fig. 14. Effect of barium on the external face of a rightside-out patch. In the control recording, the pipette contained (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes (pH 7.2). The calcium concentration was 10^{-7} M (buffered with EGTA). The bath contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.4). Barium 1 or 5 mM was added to the bathing solution. C indicates the closed state of the channel. The pipette holding potential was −20 mV with respect to the bath.

have corresponded to the flow of K^+ ions through the channel, since Cl^- ions were equilibrated and Na^+ currents would have given downward deflections. Decreasing the voltage from 0 mV to −60 mV increased the current amplitude, but did not change the opening time. The main characteristic of this channel was its flickering appearance during one burst of activity. The current-voltage relationship represented in Fig. 13B was linear over the range of +40 to −60 mV applied voltage. The reversal potential was +56 mV for a K^+ Nernst potential of +83 mV leading to a $P[K]/P[Na] = 14$. The average conductance of the channel was 28 pS. Additional experiments were also performed on rightside-out patches with KCl solution in the pipette and 35 KCl solution in the bath. Under these conditions, the current reversal potential shifted to −36 mV for a K^+ Nernst potential of −35 mV. The conductance increased to 32 pS. In symmetrical KCl solution, the current reversal potential was not different from 0 mV and the conductance was 42 pS. The effect of barium was tested on rightside-out patches at a holding potential of −20 mV. Only a high Ba^{2+} concentration induced a decrease in the opening probability of the channel (Fig. 14). Apamin reduced the channel activity when applied to the extracellular face of rightside-out patches. This is illustrated in Fig. 15 in which it can be seen that the addition of 5 10^{-7} M apamin caused a decrease in channel activity of about 90% without modification of the current amplitude. The action of the venom was completely reversible and was observed in two excised patches.

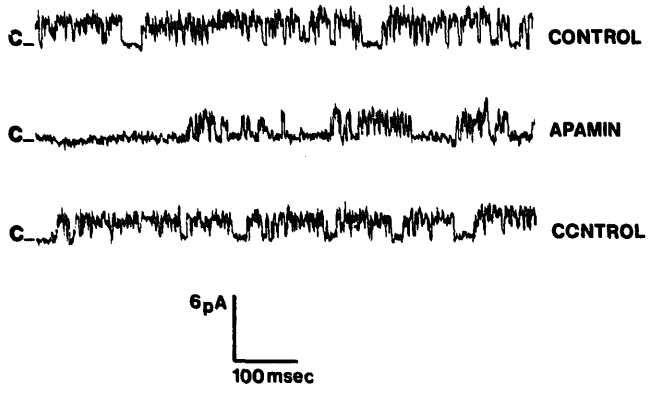


Fig. 15. Effect of apamin (10^{-7} M) on channel activity recorded in a rightside-out patch. In the control recording, the pipette was filled with (in mM): 140 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes (pH 7.2). The calcium concentration was 10^{-7} M (buffered with EGTA). The bath contained (in mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes (pH 7.4). In the middle recording, apamin was added to the bathing solution. In the lower recording, the bath was rinsed with the control solution. C indicates the closed state of the channel. The pipette holding potential was 0 mV with respect to the bath.

Discussion

In order to analyze the electrical properties of the apical membrane of the proximal tubule, we undertook primary cultures of the S1 segment isolated by microdissection. The cells grown on collagen-coated Petri dishes in a hormonally defined medium retain the ultrastructural, immunological and biochemical characteristics of the epithelium in vivo for at least 30 days after seeding as described in two previous publications [6,7].

Single-channel analysis performed on the apical membrane of cultured proximal cells revealed the existence of two different K^+ channels. One of these, observed in 20% of the patches, was highly selective to potassium and its conductance was around 200 pS in symmetrical KCl solutions. This high-conductance K^+ channel shows many of the properties of the maxi K^+ channel found in rat skeletal muscle [11], chromaffin cells [9], frog neuron [8] and rabbit T tubules [19]. The opening probability increased with the membrane potential. In cell-attached experiments this corresponds to an activation of the channel when the membrane is depolarized. However, in cell-attached patches, we were not able to determine the exact slope of the activation curve, because applying a voltage larger than −100 mV broke the patch. A sigmoidal relationship between membrane potential and opening probability was seen in the excised patch experiments in which Ca^{2+} and pH were varied. Ca^{2+} and pH sensitivities of this channel are also typical features of the maxi K^+ channel irrespective of the tissue in which it is recorded [9,10,20]. We have demonstrated a relationship between Ca^{2+} and voltage and between pH and voltage. Thus, at a given cytoplasmic Ca^{2+} concentration, the channel opened more often and for longer periods when the membrane was depolarized. Similarly, at a given pH, the opening probability was enhanced when the membrane was depolarized. These observations have also been reported by others. Notably, a very detailed study was made by

Christensen et al. [20] on the choroid plexus of amphibians. In agreement with this work we also found that the action of Ca^{2+} leads to an increase of the mean open time and a decrease of the mean closed time of the channel, whereas H^+ had the reverse effect. Finally, these combined results confirm that there is no intrinsic voltage dependence of the channel, but rather a modulation by the membrane potential of the binding of Ca^{2+} to an activation site. They also indicate that H^+ may compete with Ca^{2+} for this binding site [20]. However, the high calcium concentrations needed to activate the channel at physiological voltages in excised patches may indicate that the process of excision could result in the loss of some intracellular factors which modulate the calcium sensitivity of the channel.

In the present work, we have shown that several substances can block the K^+ channel. Of these, Ba^{2+} was very effective. Its action, reported in a wide variety of tissues, is not really specific to a given type of K^+ channel. The influence of Ba^{2+} on maxi K^+ channels was studied in the apical membranes of cultured medullary thick ascending limb cells by Guggino et al. [21]. Our data agree with their observations. The voltage dependence of the Ba^{2+} blockage is also in accordance with the known Ba^{2+} actions on K^+ channels [22,23]. It must be stressed that in our kinetic study we applied Ba^{2+} to the external side of the membrane. This could explain why we did not observe significant effects below 100 μM Ba^{2+} and were forced to use high Ba^{2+} concentrations. It has in fact, been shown that the sensitivity of the K^+ channel to Ba^{2+} is strongly dependent on the side of application [23]. To characterize the K^+ channel further, we tested the effect of crude *L. quinquestriatus* venom. The venom exerted an inhibitory effect only when applied on the external face of the membrane. Although we did not purify the toxin, it is probable that the blockage of the channel activity was due to the presence of charybdotoxin. This toxin is the main blocking agent of the maxi K^+ channel in scorpion venom [24]. Moreover, Guggino et al. [22] reported an inhibition profile with purified charybdotoxin, which was very similar to that which we obtained in the present study. Unlike Ba^{2+} and charybdotoxin, tetraethylammonium and quinidine were found to be fast blockers of the maxi K^+ channel [22]. We again found that these classical blockers decreased the channel activity: in the case of tetraethylammonium when applied to the external face and in the case of quinidine applied to the internal face. Unlike scorpion venom, apamin caused no decrease in channel activity. This toxin, extracted from bee venom, is a selective blocker of Ca^{2+}-dependent K^+ channels in neuroblastoma cells [25] and muscle [26], but does not act on maxi K^+ channels [22].

During our experiments, we observed a second K^+ channel with a lower conductance. This channel was only rarely recorded (n = 4) and never in cell-attached configuration experiments. For this reason, we at first considered that it might represent a s&conductance state of the maxi K^+ channel. However, it was not blocked by tetraethylammonium applied externally, even at a concentration as high as 10 mM, nor by scorpion venom, but was very sensitive to apamin. Its conductance was small, ranging from 28 to 42 pS. The channel was very selective for K^+ over Na' and Cs^+ (data not shown) and its activity appeared to be independent of membrane potential. Although we have not yet studied the effects of Ca^{2+}, the channel shows a strong resemblance to the K^+ channels described in cultured muscle [26].

The results of the present work demonstrate the existence of a Ca^{2+}-dependent K^+ channel of large conductance in the apical membrane of the proximal tubule in primary culture. We also identified a small conductance K^+ channel the exact pharmacology of which remains to be clarified. To our knowledge, this is the first time that such channels have been recorded in this structure. A survey of the literature concerning the mammalian kidney revealed that high conductance K^+ channels have been observed in the apical membranes of rabbit [2] and rat [4] cortical-collecting tubules. A maxi K^+ channel has been described in cultured cells of the rabbit medullary thick ascending limb [21], in primary cultures of the principal cells of cortical-collecting ducts [27] and in chick kidney cells [28]. We are at present characterizing apical channels in primary cultures of cortical ascending limb, convoluted distal bright and cortical-connecting tubule. Preliminary results suggest the presence of a K^+ channel of large conductance (92, 138 and 125 pS) [29,30]. Maxi K^+ channels are also present in cultured renal cell lines, such as MDCK [31] or JTC 12 P3 [32]. In fact, it seems that maxi K^+ channels are apically localized along most of the nephron segments. The physiological role of such a channel would necessarily vary according to the nature of the nephron segment. Along the distal segments, it could be implicated in the mechanism of K^+ secretion [2]. The most probable role of the channel along the S1 proximal tubule would be the maintenance of membrane potential during electrogenic Na^+/solute cotransport. This hypothesis is supported by the results of Brown et al. [33] who showed that actively transported sugars and amino acids increased the K^+ permeability of isolated enterocytes, probably by activating a Ca^{2+}-dependent K^+ channel.

As far as K^+ channels of smaller conductance are concerned, the data reported in the literature are more scatterer',. From voltage sensitivity and Ca^{2+}-dependence characteristics, it would seem that several types of small K^+ channel may exist [34,35]. For instance, Gogelein and Greger [34] reported a weakly voltage-dependent Ca*+-insensitive K^+ channel of 20-40 pS conductance in the basolateral membrane of the straight

proximal tubule. Parent et al. [35], found a poorly voltage-dependent, but Ca^{2+}-activated K^+ channel in the proximal convoluted tubule. Whether the apical small K^+ channel that we reported corresponds to that described by Gogelein and Greger or to that described by Parent et al. remains to be established. Moreover, the existence of the same type of channel both in the apical and the basolateral membrane would be surprising in a polarized epithelium. Thus, the question arises of the extent to which proximal tubule cells may lose part of their functional polarity when grown on collagen-coated Petri dishes. We have previously shown that such a culture process did not pertubate the distribution of the main specific hydrolases of the brush-border membrane [6,7]. On the basis of this observation, it could be reasonably supposed that the channel-constituting proteins were rightly inserted into the expected membrane during epithelial growth.

Acknowledgement

The technical assistance of L. Othmani and M. Gastineau is gratefully acknowledged.

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