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Incorporation into a planar lipid bilayer of K channels from the luminal membrane of rabbit proximal tubule

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The presence of ionic channels at the apical membrane of rabbit proximal tubule cells was investigated by fusion of brush-border membrane vesicles (BBMV) with a planar lipid bilayer (PE/PC, 1:1). The BBMV obtained from native membranes showed poor fusogenic properties. The probability of vesicles fusion with a planar bilayer was, however, enhanced by preincubating the BBMV with liposomes made of azolectin. We report here the presence in BBMV preparations of two K^+-selective channels of 65 pS and 40 pS, respectively, in asymmetrical 200//50 mM KCl solutions. The channel of 65 pS appeared highly selective to K^+ over Na^+ and Cl^- ions, while the 40 pS channel discriminated poorly between K^+ and Na^+ with a permeability ratio $P_K/P_{Na} = 4$. The open probability P_o of both channels was found to be voltage-independent within the potential range -60 mV to $+60$ mV. These K^+ channels may be related to channels identified using other methods.

Introduction

It is now generally recognized that ionic channels play a key role in the overall ion transport properties of kidney proximal tubule (PT) cells. Results obtained from electrophysiological patch clamp studies have already confirmed the presence of ionic channels, in particular of K^+-selective channels, at the basolateral membrane of several mammalian and amphibian PT preparations [1–5]. The various roles attributed to these channels include the recycling of K^+ ions in response to an increase in K^+ influx at the basolateral membrane caused by the Na^+/K^+-ATPase, and/or the creation of an efflux pathway for K^+ ions during cell volume regulation [2,5–7]. Ionic channels have also been identified at the luminal membrane of PT cells. In most cases, the results reported were obtained from patch clamp experiments carried out on established cultured cell lines or on primary cultures of PT cells. This approach led to the identification of Ca^{2+}-activated K^+ channels of large conductance in JT 21 [8] and primary cultures of rabbit PT cells [9–11], and of a 22 pS non-selective channel in cells from the OK epithelial cell line [12]. A cationic channel and a voltage-insensitive K^+ channel of 23 pS and 42 pS, respectively, were also reported by Mérot et al. [11,13] in primary cultured rabbit PT cells. In a few studies, the patch clamp technique was used directly on intact tubule preparations. For instance, a Ca^{2+}-activated K^+ channel of small conductance was identified at the luminal membrane of cut-open PT of *Necturus* [3], and K^+ channels of 35 pS and 60 pS were observed by Gögelein and Greger [14,15] at the luminal membrane of isolated perfused proximal straight tubules of mouse and rabbit kidney. Recently, Filipovic et al. [16] have shown the presence of a stretch-activated cationic channel at the apical membrane of *Necturus* PT cells. Although the physiological role of these channels remains to be established, it is generally proposed that apical K^+ channels may be involved in internal K^+ homeostasis, as well as in the control of the negative electrochemical driving force acting on Na^+-dependent transport systems [4,8,17].

An elegant alternative approach to the patch clamp methodology on PT cells is the incorporation into a planar lipid bilayer of channel proteins present in vesicles prepared from native membranes. This technique has already been used on brush-border membrane vesicles (BBMV) by Marom et al. [18] who showed the presence of a 60 pS cation-selective channel ($P_K/P_{Na} = 1.1$) at the apical membrane of rat PT cells. Evidence was also provided in this case for a Na^+-selective channel of 15 pS measured in 300 mM

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NaCl conditions. More recently, Zweifach et al. [19] identified by means of the incorporation into a planar bilayer technique a Ca^{2+}-activated K^+ channel of 250 pS (250 mM KCl) in BBMV of rabbit kidneys. They mentioned in addition the presence of three different anion-selective channels of 450, 120 and 70 pS, respectively. Because this experimental approach allows circumvention of problems due to the poor accessibility of the patch pipette to the luminal membrane in mammalian tubule preparations, without the potential lost of specificity related to the de-differentiation of cells in primary culture, we used this technique to characterize some of the channels present at the apical membrane of PT cells in rabbit kidney. Our results indicate that the BBMV prepared from rabbit renal cortex contain at least two voltage-independent K^+-selective channels of 40 and 65 pS in 200 mM KCl solutions.

Materials and Methods

Preparation of brush-border membrane vesicles from proximal tubule cells

The vesicles preparation procedure was essentially that reported by Maenz et al. [20] which is a modification of the method described by Schmitz et al. [21] and Hopfer et al. [22]. Briefly, the cortex dissected from kidneys of New Zealand white rabbits were homogenized in a 5% (w/v) 50 mM imidazole-acetate buffer (pH 7.0) containing (in mM): 300 mannitol, 5 KF, 5 ethyleneglycol bis(β-aminethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1 dithiothreitol and 1 mg/ml bovine albumin (all these products were purchased from Sigma). The frozen blender was used at full speed for 1 min. $MgCl_2$ was added to the homogenate to a final concentration of 10 mM and the solution stirred for 12 min at 4°C. This homogenate was centrifuged at $8000 \times g$ for 15 min in order to precipitate the Mg-complexed membranes. The supernatant was centrifuged at $20000 \times g$ for 30 min. A supplementary purification was made when the pellet was suspended in 250 mM sucrose, 10 mM (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (Hepes)-KOH (pH 7.4). Homogenization of the pellet was performed with 10 strokes of a motor-driven Teflon pestle. This suspension was centrifuged at $2000 \times g$ for 15 min. The pellet was discarded and the supernatant was centrifuged at $30000 \times g$ for 15 min. The pellet was resuspended in a 250 mM sucrose plus 10 mM Hepes-KOH buffer (pH 7.4) to yield a final protein concentration of 20–30 mg/ml. The pellet was aspirated through a 26 or 30 gauge needle to promote the formation of vesicles. If necessary, BBMV were stored in liquid nitrogen and aspirated through a 26 or 30 gauge needle prior to use.

Enzymatic activities

The specificity of the vesicle preparation was determined by measuring the activity of the brush-border membrane (BBM) enzymes alkaline phosphatase (AP), γ-glutamyl transpeptidase (γ-GT) and trehalase on three different vesicle preparations using standard techniques [23–25]. An enrichment factor of 7–9-fold for the AP, γ-GT and trehalase in BBMV compared to the cortical homogenate was obtained. The Na^+/K^+-ATPase activity [26] showed an enrichment factor of 1.6. These results correlate quite well with those reported by Hilden et al. [27] and by Molitoris et al. [28].

Planar lipid bilayer formation

The planar lipid bilayers were formed at room temperature from a 1:1 lipid mixture of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) purified from bovine brain (Avanti Polar Lipids, Birmingham, AL). The final lipid concentration was 25 mg/ml in decane. BLM were formed in a 200–400 μm diameter hole drilled in a delrin cup. The outline of the hole was coated with squalene before the application of the lipid mixture using a Teflon stick. Membrane thinning was monitored by applying a triangle wave of ±20 mV at the frequency of 1 Hz. The BBMV were added in the proximity of the bilayer to the chamber referred as the cis compartment. The potential was applied to the trans chamber (5 ml) with the cis compartment (3 ml) maintained at ground. Fusion was enhanced by stirring the cis side while applying a potential of −30 mV across the bilayer. In order to increase the fusion rate, the osmolarity in the cis was higher than in the trans chamber. All KCl and NaCl solutions were buffered at pH 7.4 with 10 mM Hepes-KOH or NaOH, respectively.

Vesicle fusion

Native BBMV prepared as described in Materials and Methods showed poor fusogenic properties. We attribute this particular behavior to their lipidic composition. The transbilayer distribution of phospholipids of PT apical membranes is highly asymmetrical, sphingomyelin accounting for 75% of the external leaflet with PC, PE and phosphatidylserine (PS) plus phosphatidylinositol representing 15, 7 and 3%, respectively [29]. It appears therefore that almost 90% of the phospholipid of the outer leaflet of the BBM have choline as terminal polar head group. After several unsuccessful attempts to induce the fusion of BBMV using various BLM phospholipid compositions (phosphatidylglycerol (PG), PE/PS, PE/PG, PC/PS, PE/PC at various molar ratios) we chose to use the neutral PE/PC 1:1 ratio. We tried without success to promote the fusion of the BBMV to the BLM by adding polyethylene glycol (PEG), urea or by increasing concentrations of divalent cations (Ca^{2+}, Mn^{2+}, Mg^{2+}) in the cis chamber. Good results were obtained when the BBMV were incubated with spermine, a polycation that can fix on the polar head group of glycolipid-containing lipo-

somes [30]. As gangliosides are among the external lipids of the BBMV [31], an effect of spermine was expected. The use of spermine for incorporation experiments into a BLM increased however the ratio of unstable BLM after a fusion event. Because of this effect, this procedure was not considered.

Finally, reproducible results were obtained when native BBMV were incubated 12 h at 4°C with artificial liposomes made of azolectin (L-α-phosphatidylcholine from soya bean, type II-S, Sigma). In most experiments the azolectin was purified by acetone precipitation according to Kagawa and Racker [32] in order to avoid channel activity coming from crude azolectin [33]. Azolectin was dispersed at a concentration of 10 mg/ml in 250 mM sucrose solution buffered with 10 mM Hepes-KOH at pH 7.4. The dispersion was sonicated with a large tip sonicator (Biosonik III, 40% duty cycle) till it became clear [34]. Controlled experiments carried out with a sonicated dispersion of azolectin vesicles (10 mg/ml) failed to show current fluctuations in formed BLM.

Incubation of brush-border membrane vesicles with unilamellar vesicles

The procedure of incubation of azolectin unilamellar vesicles (UV) and BBMV was adapted from Barsukov et al. [35] and Mütsch et al. [36]. The sonicated UV were centrifugated at $30\,000 \times g$ for 30 min to remove remaining multilamellar vesicles and titanium particles coming from the sonicator probe. The UV were then incubated 12 h at 4°C in the presence of BBMV. The lipid ratio UV/BBMV was approximately 100:1. The treated BBMV were separated from the UV by centrifugation at $30\,000 \times g$ for 15 min. The resulting pellet was then dispersed in the BBMV preparation buffer. A protein concentration analysis (BCA Protein Assay Reagent, Pierce Chemical Company) performed on the treated BBMV showed that almost 80% of the preincubated vesicle proteins were recovered.

Single channels recording and analysis

Ionic currents were recorded using a custom designed amplifier [37] and stored on a video cassette recorder (Sony SL 300) after digitalization (Neuro-Corder model DR-384, Neuro Data Instruments Corp.). Current recordings were played back and filtered at 100–250 Hz by means of two 4-pole Bessel filters connected in series before being sampled for computer analysis on a Panama 386. The open channel probability (P_o) and the unitary jump amplitude were derived from amplitude histogram analysis as described in details elsewhere [38]. Instantaneous single-channel I/V curves were obtained from a triangular voltage ramp of ±60 mV applied to the trans compartment at a frequency of 20 mHz. Corrections for current leaks were made during computer analysis using a multilinear interpolation procedure.

Open and closed intervals were measured from current transitions detected according to a two reference level procedure. The threshold current values I_1 and I_2 were computed for a confidence ratio α equal to 98 with

$$\alpha = \frac{P(\text{open}/I \in I_1, I_1 + \mathrm{d}I_1)}{P(\text{closed}/I \in I_1, I_1 + \mathrm{d}I_1)} \tag{1}$$

and

$$1/\alpha = \frac{P(\text{open}/I \in I_2, I_2 + \mathrm{d}I_2)}{P(\text{closed}/I \in I_2, I_2 + \mathrm{d}I_2)} \tag{2}$$

where $P(\text{open}/I \in I_i, I_i + \mathrm{d}I_i)$ and $P(\text{closed}/I \in I_i, I_i + \mathrm{d}I_i)$ represent, respectively, the probability for the channel to be open or closed knowing that the current I is within the current range $(I_i, I_i + \mathrm{d}I_i)$ $(i = 1, 2)$. For a given α, I_1 can be obtained from

$$\ln(\alpha\sigma_o P_c / \sigma_c P_o) = (I_i - I_c)^2/2\sigma_c^2 - (I_i - I_o)^2/2\sigma_o^2 \quad (i = 1) \tag{3}$$

where I_o, σ_o^2 and I_c, σ_c^2 are the mean values and variances estimated for the Gaussians corresponding to the current amplitude distribution for the open and closed state, respectively, and P_o, P_c the open and closed channel probabilities (see Appendix I).

Current amplitude histograms and time-interval analysis were both used and tested for internal consistency in experiments where only one channel was present. Time intervals < 0.5 ms were usually not included in our calculations.

Results

The 65 pS channel

Fig. 1 shows a series of current records measured at different holding potentials following the fusion of PT luminal membrane vesicles in 200 mM (cis)//50 mM (trans) KCl conditions. Downwards current jumps corresponding to channel openings are observed for voltages ranging from −60 mV to +30 mV. The amplitude of the current jumps decreased as the applied potential increased to more positive values and reversed at a potential equal to +30 mV. For applied voltages more positive than +30 mV, the channel openings appeared as upward deflections of increasing amplitude. The instantaneous I/V curve of this channel measured in 200 mM (cis)//50 mM (trans) KCl conditions is illustrated in Fig. 2A. The channel I/V curve shows a slight rectification with slope conductances of 65 pS and 40 pS for negative and positive currents, respectively. The zero current potential was

estimated at +30 mV, a value close to the Nernst equilibrium potential for the K^+ ions. This result indicates that the channel illustrated in Fig. 1 is mainly permeable to cations since a Cl^- permeable channel would have resulted in a Nernst equilibrium potential of −30 mV under the prevailing experimental conditions. Similar recordings were obtained in four different vesicle preparations.

The channel selectivity was further investigated in a series of experiments in which 100 mM NaCl was added to the trans compartment in 200 mM (cis)//50 mM (trans) KCl conditions. A representative instantaneous *I/V* curve is illustrated in Fig. 2B. As observed, the reverse potential in both *I/V* curves remained constant despite the presence of 100 mM Na^+ ions (Fig. 2B) in the trans chamber. On the basis of this result, it is concluded that the 65 pS channel we identified is highly selective to K^+ ions.

Fig. 3A illustrates the effect of voltage on the open channel probability measured from two sets of experiments in 200 mM (cis)//50 mM (trans) KCl conditions

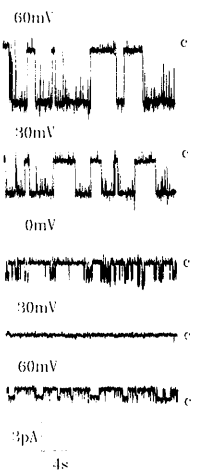

Fig. 1. Single-channel activity measured at various potentials following fusion of BBM vesicles of PT cells with a planar lipid bilayer of PE/PC (1:1) in 200 mM (cis)//50 mM (trans) KCl solution. The current level corresponding to the closed state is indicated by the letter c. Zero current was obtained at +30 mV, a value close to expected Nernst equilibrium potential of K^+ ions. Current records were filtered at 250 Hz.

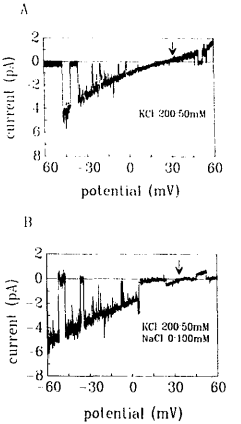

Fig. 2. Instantaneous *I/V* curves of the 60 pS channel measured in (A) 200 mM (cis)//50 mM (trans) KCl solutions and in (B) 200 mM KCl, 0 NaCl (cis)//50 mM KCl, 100 mM NaCl (trans) solutions. In both cases, the Nernst equilibrium potential value, E_r, indicated by an arrow, is approximatively equal to +33±4 mV. This result indicates that the 60 pS channel is highly K^+ selective. Both current voltage ramps were filtered at 100 Hz.

over 60 s recording periods for each voltage. The results indicate that the open channel probability is nearly voltage-independent within the voltage range −60 mV to +70 mV.

The channel kinetics behaviour was further investigated by means of an analysis of the channel open and closed time interval distribution carried out on continuous 60 s long single-channel records. The time interval distributions were computed according to the method described by Sigworth and Sine [39] in which the distribution of dwell times is plotted with a logarithmic time axis. The results presented in Fig. 3B indicate that the channel open and closed time interval distributions can be fitted by at least two exponential functions. The distribution (S_1) of open dwell times is characterized by an important contribution (30–40%) of intervals within the 100 ms time range with fast events of 2 ms mean value representing 60–70% of the total number of intervals detected. The closed time interval distribution (S_2) shows in contrast fewer (5–10%) long intervals (1 s mean value) and a more important fraction of events within 1 ms time range (90–95%). Because the mean value of the slow closed

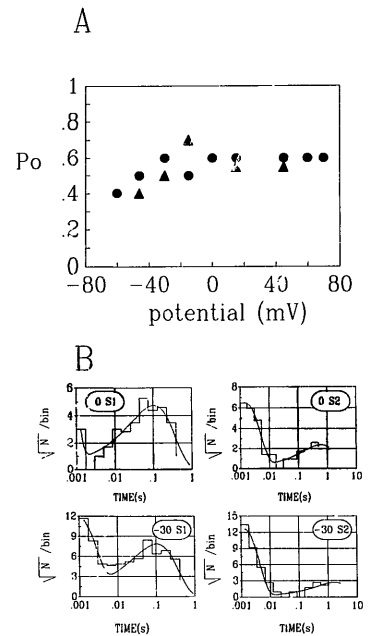

Fig. 3. (A) Effect of voltage on open channel probability. P_o was measured from current records longer than 45 s obtained from two different sets of experiments. The value of P_o appeared slighly voltage-dependent within the voltage range −60 mV to +70 mV. (B) Open (S_1) and closed (S_2) time interval distributions measured at 0 and −30 mV. Results obtained using 5 bins per decade. Open and closed time interval distributions could be fitted by a summation of two exponential functions. Data acquisition was made at 10000 points per second.

time interval distribution is 500 to 1000 times more important than that computed for the fast closed time interval distribution, long intervals will therefore contribute quite significantly to the overall channel open probability despite their low occurrence. Finally, we failed to block the channel by the addition of Ba^{2+} to the trans chamber at concentration up to 12 mM (data not shown).

The 40 pS channel

In other experiments ($n = 5$) we observed single channel events not related to the 65 pS K^+ channel described previously. Representative single-channel records measured in asymmetrical 200 mM (cis)//50 mM (trans) KCl conditions at various voltages are shown in Fig. 4A (left panel). In contrast to the channel activity presented in Fig. 1, the fluctuation pattern in this case is characterized by the appearence of flickering current bursts. Channel openings appeared as downward current deflections for potentials less than +30 mV with upward channel openings at +45 mV. The current amplitude probability density was estimated for each of the current records shown in Fig. 4A. The probability density function could in all cases be fitted by a summation of two Gaussians as shown on Fig. 4A (solid line, right panel). The effect of voltage on P_o is illustrated in Fig. 4B. The channel was found to be slighly voltage sensitive with P_o varying from 0.4 to 0.6 over the voltage range of −60 mV to +60 mV. The instantaneous I/V curve measured in 200 mM (cis)//50 mM (trans) KCl ionic conditions is illustrated in Fig. 5A. The channel conductance measured for negative currents was estimated at 40 pS. The reversal potential computed either from the current records obtained at constant potentials (fig. 4A) or from instantaneous channel I/V curves (Fig. 5A) corresponds in both cases to a value close to +30 mV. A zero current potential of +30 mV under the prevailing ionic conditions indicates that this channel is selectively permeable to cations.

The channel cationic selectivity was subsequently investigated in experiments where single channel I/V curves were measured in 200 mM (cis)//50 mM KCl + 150 mM NaCl (trans) ionic conditions (Fig. 5B). As observed, the presence of 150 mM Na^+ ions in the trans chamber resulted in a 13 mV negative shift in reversal potential to a value approximately equal to +15 mV. On the basis of the Goldmann-Hodgkin-Katz equation, this result led to a ratio P_K/P_{Na} of 4. In addition, the channel conductance for negative currents in the presence of Na^+ remained unchanged.

Discussion

Brush-border membrane vesicle fusion

The purpose of this study was to investigate the presence of ionic channels at the luminal membrane of PT cells using the fusion of BBMV with a BLM. As mentioned earlier in Materials and Methods BBMV showed poor fusogenic properties when the standard incorporation technique which consists of maintaining the vesicle containing compartment hyperosmotic with respect to the trans chamber was used [40]. A different experimental approach was used by Zweifach et al. [19] who succeeded to induce fusion of native BBMV by

maintaining the intravesicular medium hyperosmotic in regard to the cis chamber and the trans chamber hyperosmotic in regard to the cis compartment. Their experimental conditions include the presence of a high concentration of Ca^{2+} in the incubation medium. In the present study, improved fusion rate was obtained

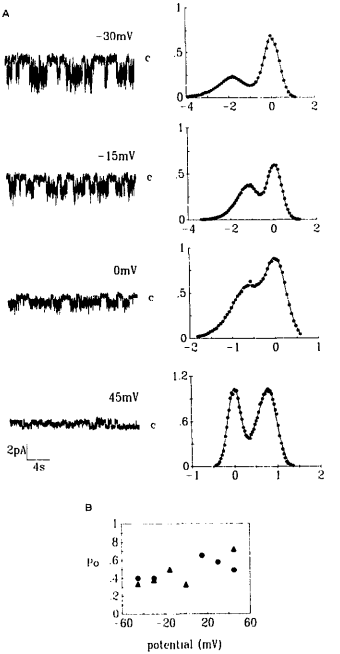

Fig. 4. (A) (Left panel) Single-channel records measured at four different holding potentials in 200 mM (cis)//50 mM (trans) KCl solutions. The current reverse potential was estimated at +30 mV, indicating a cation selective channel under the prevailing ionic conditions. The amplitude histograms measured for each of current records are shown on the right panel with the theoretical predictions coming from the summation two Gaussian functions (solid line). Current records were filtered at 250 Hz. (B) The open probability, P_o as a function of voltage for the 40 pS channel in the absence (●) and the presence of NaCl 150 mM (▲) in the trans compartment. In both cases, the KCl conditions were 200 mM (cis)//50 mM (trans). P_o values were computed from current records longer than 45 s. The current records were filtered at 250 Hz.

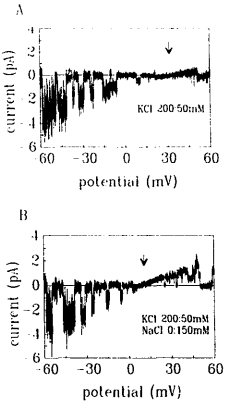

Fig. 5. Selectivity of the 40 pS channel. Intantaneous I/V curves were measured in 200 mM (cis)//50 mM (trans) KCl solutions and in (B) 200 mM KCl, 0 NaCl (cis)//50 mM KCl, 150 mM NaCl (trans) solution. Reversal potential in each case is indicated by an arrow. The relative position of the arrow shows the shift in reversal potential due to the presence of Na^+. A negative shift of 13 mV (from 28±2 to 15±2 mV) observed in the presence of 100 mM Na^+ (trans) indicates a permeability ratio of P_K/P_{Na} of 4 as determined from the GHK equation. Instantaneous voltage ramps were filtered at 100 Hz.

with azolectin pretreated BBMV. The exact nature of the azolectin effect remains to be established, but it is likely that azolectin caused a modification of the BBMV lipid composition which facilitates membrane fusion. In this regard, Mütsch et al. [36] have shown a net transfer of lipids from PC liposomes into intestinal BBMV (ratio 100:1). Experiments with azolectin liposomes alone did not show single-channel activity. The channel events measured in the presence of treated BBMV are likely therefore to reflect the contribution of ionic channels present at the luminal membrane of PT cells and not that of contaminants coming from the azolectin [33]. In addition, the fact that most of our experiments did not show single-channel events despite the use of treated vesicles rules out a systematic contribution coming from a component in the azolectin extract.

Comparison with K^+ channels found at the apical membrane of proximal tubules

In four different vesicle preparations, we observed a highly K^+-selective channel of 65 pS in asymmetrical

200 mM (cis)//50 mM (trans) KCl solutions characterized by a voltage-independent open channel probability. A K^+ channel of 60 pS was identified by Gögelein [15] at the luminal membrane of the proximal straight tubule in mouse kidney. A 60 pS channel highly selective to K^+ ions ($P_K/P_{Na} = 32$) was also found by Kawahara et al. [13] at the apical membrane of *Necturus* PT cells. This channel was activated by membrane depolarization (e-fold for 41.7 mV) and appeared sensitive to internal Ca^{2+}. Such a strong voltage dependency was not observed in the incorporation experiments described in this work. Using the incorporation into planar bilayer technique, Marom et al. [18] have reported a cationic channel of 60 pS in 300 mM NaCl ($P_K/P_{Na} = 1$). In contrast to the results shown in Fig. 2B, this channel did not discriminate between K^+ and Na^+ ions. It cannot be excluded, however, that the 60 pS channel observed by Marom et al. [18] in 300 mM NaCl corresponds to the 40 pS channel described here if one takes into account the difference in ionic concentrations used in both studies. Finally, the 60 pS channel obtained by incorporation can not be regarded as one of the maxi K^+-Ca^{2+} channels reported at the apical membrane on several PT preparations [9–11,19]. In addition to a high conductance (200 pS), the maxi K^+-Ca^{2+} channels showed on the average an e-fold increase in P_o per 12 mV depolarization which is in desagreement with the results presented in Figs. 1–3 in this work.

A K^+-selective channel of 40 pS in 200 mM (cis)//50 mM (trans) KCl conditions was recorded in five different vesicle preparations. A ratio P_K/P_{Na} equal to 4 was measured in this case and the open channel probability appeared voltage-independent. A voltage-insensitive K^+ channel of 42 pS measured in symmetrical 140 mM KCl was observed by Merot et al. [11] at the luminal membrane of cultured PT cells using the patch clamp method. The permeability ratio P_K/P_{Na} in this case was equal to 14, a value higher than that obtained for the 40 pS channel described in this work. A cationic channel of 33 pS in 140 mM KCl was found by Gögelein and Greger [14] at the luminal membrane of rabbit kidney, but no detailed description of the channel characteristics was provided. A stretch-activated cationic channel was characterized by Filipovic and Sackin [16] at the luminal membrane of *Necturus* PT cells. The reported ratio P_K/P_{Na} of 1.5 approximates the permeability ratio obtained for the 40 pS channel in the present incorporation experiments. However, the channel described here showed a better discrimination for cations over anions with a ratio $P_K/P_{Cl} > 18$ compared to $P_K/P_{Cl} = 9$ [16]. Finally, it has to be mentioned that we never observed the 65 pS and 40 pS channels present in the same current records. Assuming that the vesicles are formed from continuous membrane fragments, such a result may suggest that these K^+ channels are probably scattered as single proteins in the luminal membrane of PT cells.

Comparison with K^+ channels found at the basolateral membrane of proximal tubules

A K^+-selective channel of 54 pS measured in 200 mM KCl was observed by Parent et al. [4] at the basolateral membrane of rabbit proximal tubule using the patch clamp technique. The I-V characteristics and voltage dependency of this basolateral channel differ from that measured for the 60 pS channel obtained by incorporation. The channel reported by Parent et al. [4] can not therefore account for the experimental observations presented in this work. Furthermore the channel observed in patch clamp could not usually be maintained in an active state following membrane patch excision suggesting that this channel forming protein may not be observed under the conditions prevailing in incorporation experiments. Gögelein and Greger [1] also reported a similar 40–50 pS channel at the basolateral membrane of rabbit proximal straight tubules. A slight increase in P_o with depolarizing potentials was inferred in this case. Finally, K^+-selective channels ($P_K/P_{Na} = 11$) with conductances ranging from 30–60 pS were observed in two different studies carried out at the basolateral membrane of *Necturus* PT cells [3,41]. In both cases, the open channel probability increased significantly at hyperpolarizing voltages. On the basis of their respective P_o vs. V relationship, none of the K^+ channels reported at the basolateral membrane of PT cells seems therefore to correspond to the K^+ channels described in the present study.

Finally, contaminants from internal membrane like endosomal elements are not excluded. In the preparation reported here, we observed once, an anionic channel that corresponds in conductance and in kinetic behavior to the 80 pS chloride channel characterized in endosomes [42]. Endosomal organelles should not however contribute significantly to the observed single channel activity since no cationic channel has been reported to be present in these structures [42].

Conclusions

It is likely that the channels reported in this work are from the apical membrane of PT cells but a detailed description of the cellular agents responsible for channel regulation is required in order to confirm the apical nature of these channel forming proteins. Such agents remains to be identified. As for the physiological relevance of the K^+ channels characterized, it be suggested that these channels may serve to maintain a negative cellular potential in order to insure Na^+-dependent transport processes across the luminal membrane [17]. These channels may also be involved in volume regulation [9,12,16] and in internal K^+ home-

ostasis in response to the accumulation of K^+ inside the cell following the stimulation of the Na^+/K^+-ATPase. Now that the incorporation technique can be used on BBM preparation of PT cells, it is hoped that more information on the physiological role of these structures will soon become available. Work along this line is currently in progress.

Appendix I

Let

$$\alpha = \frac{P(\text{open}/I \in I_1,\ I_1 + \mathrm{d}I_1)P(I_1)\mathrm{d}I_1}{P(\text{closed}/I \in I_1,\ I_1 + \mathrm{d}I_1)P(I_1)\mathrm{d}I_1} \qquad \text{(A-1)}$$

then

$$\alpha = \frac{P(I \in I_1,\ I_1 + \mathrm{d}I_1/\text{open})P_o}{P(I \in I_1,\ I_1 + \mathrm{d}I_1/\text{closed})P_c} \qquad \text{(A-2)}$$

where P_o, P_c are the open and closed channel probabilities. Assuming that the current amplitude distribution $P(I)\mathrm{d}I$ is given by

$$P(I)\mathrm{d}I = \left[\frac{P_o \exp(-(I-I_o)^2/2\sigma_o^2)}{\sqrt{2\pi}\sigma_o} + \frac{P_c \exp(-(I-I_c)^2/2\sigma_c^2)}{\sqrt{2\pi}\sigma_c}\right]\mathrm{d}I \qquad \text{(A-3)}$$

$P(I \in I_1,\ I_1 + \mathrm{d}I_1/\text{open})$ and $P(I \in I_1,\ I_1 + \mathrm{d}I_1/\text{closed})$ now read

$$P(I \in I_1,\ I_1 + \mathrm{d}I_1/\text{open})\mathrm{d}I = \frac{\exp(-(I_1-I_o)^2/2\sigma_o^2)\mathrm{d}I}{\sqrt{2\pi}\sigma_o} \qquad \text{(A-4)}$$

and

$$P(I \in I_1,\ I_1 + \mathrm{d}I_1/\text{closed})\mathrm{d}I = \frac{\exp(-(I_1-I_c)^2/2\sigma_c^2)\mathrm{d}I}{\sqrt{2\pi}\sigma_c} \qquad \text{(A-5)}$$

where I_o, σ_o^2 and I_c, σ_c^2 are the mean values and variances estimated for the gaussians corresponding to the current amplitude distribution for the open and closed state, respectively. Eqn. 3 follows directly by substituting the expressions A-4 and A-5 into A-2. A similar procedure can be applied to compute I_2.

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