Volume 269, number 2, 315-318 FEB 08809 September 1990

Potassium channels in basolateral membrane vesicles from pars convoluta of rabbit proximal tubule

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Received 9 July 1990

The characteristics of $^{86}Rb^+$ fluxes through conductive channels in basolateral-membrane vesicles isolated from pars convoluta of rabbit proximal tubule were investigated. In KCl loaded vesicles a transient accumulation of $^{86}Rb^+$ was observed which was inhibited by $BaCl_2$. The accumulation was driven by an electrical diffusion potential, as shown in experiments using membrane vesicles loaded with Li_2SO_4 and an outwardly directed Li^+ gradient established with a Li^+-ionophore. The vesicles containing the channel showed a cation selectivity with the order $K^+ = Rb^+ >> Li^+ \geq Na^+ > choline^+$. The $^{86}Rb^+$ flux was dependent on intravesicular Ca^{2+}. Increasing concentrations of Ca^{2+} gradually decreased the $^{86}Rb^+$ uptake.

Cation channel; Basolateral membrane vesicle; Pars convoluta

1. INTRODUCTION

Ionic channels constitute important pathways for the movement of ions across epithelial cell membranes. The luminal membrane is generally conductive for Na^+, while the basolateral membrane is almost exclusively conductive for K^+. However, in the proximal tubule there is now direct evidence for the existence of K^+ channels both in luminal and basolateral membranes [1-4]. Recently, also nonselective cation channels which discriminate poorly between Na^+ and K^+ ions have been observed in luminal membranes [5,6] as well as basolateral membranes [7-9].

In a previous paper we characterized K^+ channels in luminal membranes of rabbit proximal straight tubule (pars recta) [3]. In the present communication we describe the properties of a K^+-selective channel in basolateral membranes from pars convoluta. The channel is moderately Ba^{2+}-sensitive, but strongly inhibited by intravesicular Ca^{2+}.

2. MATERIALS AND METHODS

2.1. *Preparation of basolateral membrane vesicles*

Basolateral vesicles were isolated from the pars convoluta of the proximal tubule of rabbit kidney according to the protocol described previously [10]. In all experiments 10 μM of the Ca^{2+} ionophore A23187 and 3.75 mM EGTA were included in the washing medium of the vesicles to remove traces of intravesicular Ca^{2+} [11]. The purity of the membrane vesicle preparations was examined by electron microscopy and by measuring specific activities of various enzyme markers [12]. The amount of protein was determined as described by Lowry et al. [13], and as modified by Peterson [14], with bovine serum albumin as a standard. All solutions were filtered through 0.22 μm filters and sterilized before use.

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2.2. *Assay of Rb^+ transport*

Influx of $^{86}Rb^+$ was measured in K^+ (55 mM)-loaded vesicles, analogous to flux-measurement procedures reported previously [3,15]. Dowex AG50W-X8 columns were prepared in 1 ml tuberculin syringes. The resin was washed with 1 M Tris to convert it to the Tris form, followed by 5-6 vols of 298 mM sucrose/3.75 mM EGTA/27 mM Hepes/Tris, pH 7.4, containing bovine serum albumin (1 mg/ml). In the standard loading procedure, vesicles, 500 μl (approx. 10 mg/ml) were suspended in a buffer of 185 mM sucrose, 3.75 mM EGTA, 2 mM ouabain, 350 μM amiloride, 100 μM sodium vanadate, 55 mM KCl/27 mM Hepes/Tris, pH 7.4, for 1.5 h on ice. Loading with Li_2SO_4 was conducted in the absence and presence of 50 μM of the Li^+ ionophore *NN'*-diheptyl-*NN'*-5,5-tetramethyl-3,7-dioxanonanediamide (purchased from Fluka, Buchs, Switzerland). The vesicles were pelleted by centrifugation (25 000 × *g* for 30 min at 2°C) and resuspended in the same buffer to a protein concentration of approx. 15 mg/ml. Portions (300 μl) were applied to separate columns. The individual eluates were diluted to 1.0 ml with 298 mM sucrose/3.75 mM EGTA/27 mM Hepes/Tris buffer, pH 7.4, and 75 μl was withdrawn for protein determination. In some experiments $BaCl_2$ was added to final concentrations of 10 mM. The experiment was initiated by adding $^{86}Rb^+$ (210 kBq; 25 nmol) to the incubation medium. Samples of 145 μl were taken out at various times (1, 3, 5, 7, 10 and 15 min), and uptake was stopped by passing the samples through the Dowex AG50W-X8 Tris form columns by centrifugation. The eluates were diluted to 500 μl and used directly for radioactivity determinations. In the measurements of Ca^{2+}-dependent transport, the Ca^{2+}-depleted vesicles were incubated for 1 min at 20°C with 298 mM sucrose/27 mM Hepes/Tris, pH 7.4, containing 3.75 mM EGTA and 1.15-4.75 mM $CaCl_2$ to give the desired concentration of free Ca^{2+} before adding tracer $^{86}Rb^+$. The free Ca^{2+} in the

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00145793/90/$3.50

EGTA-Ca^{2+} buffer system was calculated according to Pershadsingh and McDonald [16].

3. RESULTS AND DISCUSSION

3.1. *Effect of* Ba^{2+}

Fig. 1A shows the time course of $^{86}Rb^+$ uptake into basolateral membrane vesicles from pars convoluta loaded with KCl (55 mM) in the absence and presence of Ba^{2+} ions. In the absence of Ba^{2+} the uptake of the isotope against the opposite K^+ concentration gradient rose to a peak value at 10–15 min. In the presence of 10 mM Ba^{2+} $^{86}Rb^+$ uptake is reduced to about 50% of the control. Fig. 1B summarizes the effect of various concentrations of Ba^{2+} on the uptake of $^{86}Rb^+$ in basolateral vesicles. It is apparent from the figure that the accumulation of isotope gradually decreases with an increasing medium concentration of Ba^{2+}. Addition of 100 mM tetraethylammonium did not change the isotope uptake significantly (not shown).

3.2. *Electrical nature of* $^{86}Rb^+$ *uptake*

The data presented in Table I provide direct evidence that $^{86}Rb^+$ accumulation is driven by an electrical potential. In these experiments basolateral membrane vesicles from pars convoluta were loaded with 55 mM KCl, 27.5 mM K_2SO_4 or 27.5 mM Li_2SO_4 and the uptake of $^{86}Rb^+$ was measured. It is apparent from the Table that the rate of uptake into both K_2SO_4 and Li_2SO_4 loaded vesicles is much higher than into KCl loaded vesicles. This is in agreement with the fact that the passive permeability of the vesicle membrane is lower for sulphate than for chloride [17]. Accordingly, the cation diffusion potential should therefore be short-circuited by anions of increasing permeability. Thus, the rate of $^{86}Rb^+$ uptake should be higher in sulphate than in chloride loaded vesicles. This was further tested by examining the effect of Li^+-ionophore on the uptake of $^{86}Rb^+$ into Li_2SO_4-loaded vesicles. Li^+-ionophore dissolved in dimethyl sulphoxide (0.5% w/v), or in parallel experiments pure dimethyl sulphoxide, was added to the incubation medium 1 min before tracer $^{86}Rb^+$. The uptake is enhanced by a factor 2.7 in the presence of the ionophore. These results demonstrate that the $^{86}Rb^+$ flux is driven by an electrical diffusion potential.

3.3. *Ion selectivity*

Fig. 2 summarizes the univalent cation selectivity of the vesicles. The selectivity to cations was examined by loading vesicles with 55 mM KCl and investigating the effect of including alkali chloride in the incubation medium in a final concentration of 10 mM. The presence of 10 mM KCl or 10 mM RbCl reduced the $^{86}Rb^+$ uptake to about 50%. The presence of LiCl or NaCl in the external medium gave $^{86}Rb^+$ uptakes of 81% and 84%, respectively. Choline chloride only sligthly affected the $^{86}Rb^+$ uptake. The most direct interpretation of these data is that addition of permeable cations to the outside medium depolarized the electrical gradient established across the channels and reducing

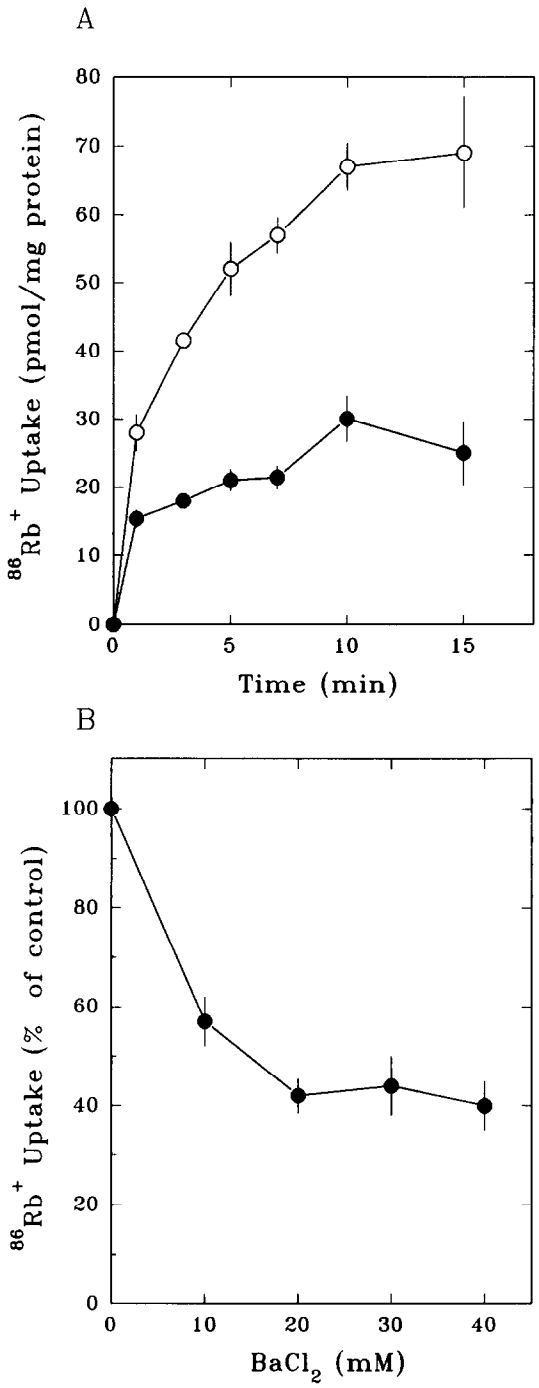

Fig. 1. (A) Time course of $^{86}Rb^+$ uptake into basolateral membrane vesicles from pars convoluta of rabbit proximal tubules. (a) One ml of vesicles (protein conc. 2.6 mg/ml), K^+_{in} = 55 mM, K^+_{out} = 0 mM, was incubated at room temperature with $^{86}Rb^+$ (19 μM) without inhibitor (open circle), and in presence of 10 mM $BaCl_2$ (closed circle). (B) shows the effect of 0–40 mM $BaCl_2$ in the incubation medium on the uptake of $^{86}Rb^+$. Means ±SE of 4 independent preparations are shown.

Table I

Demonstration of electrogenic K^+ channels in basolateral-membrane vesicles from pars convoluta

Intravesicular composition	Added to incubation medium	Relative $^{86}Rb^+$ uptake
KCl (55 mM)		100
KCl (55 mM)	$BaCl_2$ (10 mM)	48 ± 5
K_2SO_4		114 ± 13
Li_2SO_4		169 ± 27
Li_2SO_4	Li-ionophore (50 μM)	269 ± 25

The uptakes were measured for 5 min. Result are means ± SE ($n = 4$)

the driving force for intravesicular accumulation of $^{86}Rb^+$. The depolarizing effect and thus the permeability is much higher for K^+ and Rb^+ ions than for Li^+, Na^+ or choline ions. Accordingly, the cation selectivity of the channel is $K^+ = Rb^+ >> Li^+ \geqslant Na^+ >$-choline$^+$. In comparison Parent et al. [4] used the patch-clamp technique to demonstrate a K^+-selective channel in basolateral membrane of rabbit proximal tubule. By this technique they found the channel 16 times more permeable to K^+ than Na^+ ions.

3.4. *Inhibition of $^{86}Rb^+$ uptake by intravesicular Ca^{2+}*

The inhibitory effect of intravesicular Ca^{2+} on the $^{86}Rb^+$ fluxes into luminal membrane vesicles from pars convoluta of rabbit proximal tubule has been described previously [18]. In that study vesicles were prepared by Mg^{2+} precipitation and washed in medium containing 10 μM of the Ca^{2+} ionophore A23187 and 3.75 mM EGTA to remove traces of intravesicular Ca^{2+}. The desired concentration of free Ca^{2+} was achieved by incubation in a Ca^{2+}/EGTA buffer before adding tracer $^{86}Rb^+$. Exactly the same procedures were used in the present study. It appears from Fig. 3 that Ca^{2+} has a great impact on the $^{86}Rb^+$ influx. At 10 nM free Ca^{2+} the uptake is approx. 80%, and at 100 nM free Ca^{2+} the uptake is approx. the same as in the presence of 10 mM Ba^{2+}, and at 10 μM Ca^{2+} the uptake is below 20% of the maximum uptake of $^{86}Rb^+$. The present findings on the influence of intravesicular Ca^{2+} are in contrast to what was found for basolateral K^+ channels in rabbit proximal straight tubule (pars recta). These were reported not to be directly dependent on Ca^{2+} ions [19,20]. Neither K^+ channels in basolateral membrane of *Necturus* proximal tubule were affected by cytosolic side Ca^{2+} in a concentration of 3 mM [21]. Parent et al. [4] reported that a K^+-selective channel in the basolateral membrane of rabbit proximal convoluted tubule was not activated by an internal Ca^{2+} concentration as high as 2 mM. Rather they observed a run-down in K^+-channel activity under any Ca^{2+} concentration condition. However, the Ca^{2+} dependence was not studied in detail. Our results clearly demonstrate that increasing concentrations of intravesicular Ca^{2+} gradually decreased $^{86}Rb^+$ uptake.

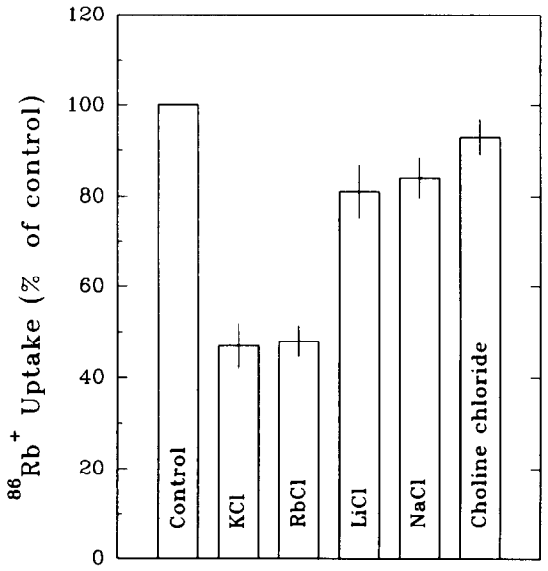

Fig. 2. The effect of external cations on the $^{86}Rb^+$ uptake into basolateral membrane vesicles from pars convoluta. The vesicles were loaded with 55 mM KCl and the alkali chloride salts were added together with tracer $^{86}Rb^+$ to the incubation medium in a final concentration of 10 mM.

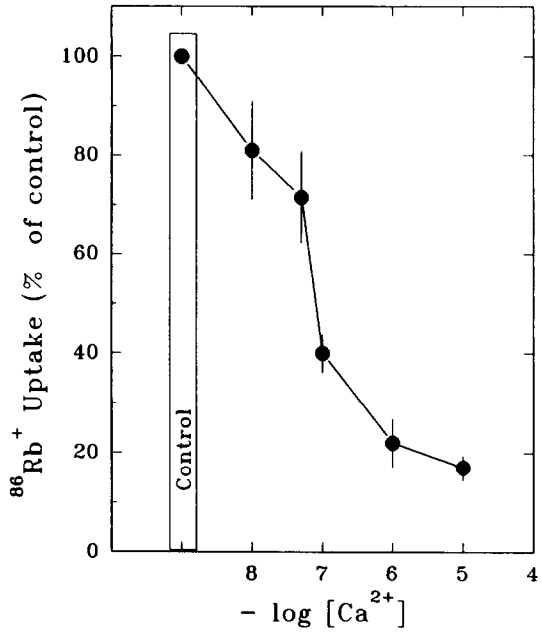

Fig. 3. The effect of Ca^{2+} on the $^{86}Rb^+$ uptake into basolateral membrane vesicles from pars convoluta. The vesicles were depleted for Ca^{2+} by treatment with 3.75 mM EGTA and 10 μM Ca^{2+} ionophore A23187 before loading the vesicles with 55 mM KCl. The vesicles were incubated for 1 min at 20°C with 298 mM sucrose, 27 mM Hepes/Tris, pH 7.4 containing 3.75 mM EGTA and 1.15–4.75 mM $CaCl_2$ to give the desired concentration of free Ca^{2+} before adding tracer $^{86}Rb^+$.

Acknowledgements: This study was supported in part by the Danish Medical Research Council, The Danish Biotechnology Programme, Aarhus Universitets Forskningsfond, P. Carl Petersens Fond, Fonden til Laegevidenskabens Fremme, Fogh-Nielsens Legat and NOVO Fond.

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