Non-selective cation channels in basolateral-membrane vesicles from pars recta of rabbit kidney proximal tubule

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The characteristics of $^{86}\text{Rb}^+$ fluxes through conductive channels in basolateral-membrane vesicles isolated from pars recta of rabbit kidney proximal tubule were investigated. In RbCl -, KCl - and NaCl -loaded vesicles a transient and almost equal accumulation of $^{86}\text{Rb}^+$ was observed. The uptakes of $^{86}\text{Rb}^+$ were inhibited to the same extent by 10 mM- BaCl_2 in all loadings. The accumulation was driven by an electrical diffusion potential. The $^{86}\text{Rb}^+$ flux was dependent on intravesicular Ca^{2+} . Increasing concentrations of Ca^{2+} gradually decreased the $^{86}\text{Rb}^+$ uptake. At 10 μM - Ca^{2+} the radioisotope flux was below 20% of control. The vesicles containing the channel showed very low selectivity among the univalent cations K^+ , Rb^+ , Li^+ , Na^+ and choline $^+$.

INTRODUCTION

Several studies have provided direct evidence for the existence of K^+ -selective channels in both luminal and basolateral membranes of renal proximal tubule (Gögelein & Greger, 1984, 1987; Kawahara *et al.*, 1987; Sackin & Palmer, 1987; Parent *et al.*, 1988; Jacobsen *et al.*, 1989a,b, 1990b). By the patch-clamp technique, non-selective cation channels which discriminate poorly between Na^+ and K^+ ions have been observed in luminal membranes of renal proximal tubule (Marom *et al.*, 1989), in basolateral membranes of proximal tubule (Gögelein & Greger, 1986) and in the thick ascending limb of Henle's loop (Teulon *et al.*, 1987; Paulais & Teulon, 1989).

Recently we characterized K^+ -selective channels in luminal membranes of rabbit proximal straight tubule (pars recta) by measuring channel-mediated $^{86}\text{Rb}^+$ flux into cell membrane vesicles (Jacobsen *et al.*, 1989a). The characterization was carried out as a first step towards purification of the proteins involved in the transport process. In the present paper we describe the properties of a K^+ channel in basolateral membranes from pars recta of rabbit proximal tubule. The channel is highly Ba^{2+} -sensitive, and is strongly inhibited by intravesicular Ca^{2+} , but has very low selectivity towards cations.

EXPERIMENTAL

Preparation of basolateral membrane vesicles

Basolateral vesicles were isolated from the pars recta of the proximal tubule of rabbit kidney by the protocol described (Sheikh & Møller, 1987). 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+} (Jacobsen *et al.*, 1988). The purity of the membrane vesicle preparations was examined by electron microscopy and by measuring specific activities of various enzyme markers (Sheikh *et al.*, 1982). The amount of protein was determined as described by Lowry *et al.* (1951), and as modified by Peterson (1977), with BSA as a standard. All solutions were filtered through 0.22 μm -pore filters and sterilized before use.

Assay of Rb^+ transport

Influx of $^{86}\text{Rb}^+$ was measured in K^+ (55 mM)-loaded vesicles, analogous to flux measurement procedures reported previously (Jacobsen *et al.*, 1989a). Dowex AG50W-X8 columns were prepared into 1 ml tuberculin syringes. The resin was washed with 1 M-Tris to convert it into the Tris form, followed by 5–6 vol. of 298 mM-sucrose/3.75 mM-EGTA/27 mM-Hepes/Tris, pH 7.4, containing BSA (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 (or 55 mM- RbCl , - NaCl , -choline chloride or 27.5 mM- K_2SO_4)/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-dioxanonanedi-*amides* (purchased from Fluka, Buchs, Switzerland). The vesicles were pelleted by centrifugation (25000 *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 on separate Dowex columns, and forced through the columns by centrifugation in a clinical centrifuge (1000 rev./min for 2 min). The eluates were pooled and 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 a final concentration of 10 mM. The experiment was initiated by adding $^{86}\text{Rb}^+$ (210 kBq; 25 nmol) to the incubation medium either as such or together with alkali salts. Samples (145 μl) were taken at various time intervals (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 (1000 rev./min for 2 min). The eluates were diluted to 500 μl and used directly for radioactivity determinations. In the measurement 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 $^{86}\text{Rb}^+$. The free Ca^{2+} in the EGTA- Ca^{2+} buffer system was calculated in accordance with Pershadsingh & McDonald (1980).

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Fig. 1. $^{86}\text{Rb}^+$ uptake into basolateral-membrane vesicles from pars recta of rabbit proximal tubule

(a) Time dependence of $^{86}\text{Rb}^+$ uptake. Vesicles ($400\ \mu\text{l}$; protein concn. $7.4\ \text{mg/ml}$; $\text{K}^+_{\text{in}} = 55\ \text{mM}$, $\text{K}^+_{\text{out}} = 0\ \text{mM}$) were incubated at room temperature with $^{86}\text{Rb}^+$ ($19\ \mu\text{M}$) without inhibitor (○) and in the presence of $10\ \text{mM}$ - BaCl_2 (●). (b) shows the effect of 0 – $40\ \text{mM}$ - BaCl_2 in the incubation medium on the $^{86}\text{Rb}^+$ uptake. The uptakes were measured for $10\ \text{min}$. Maximum uptake (control) was $200 \pm 13\ \text{pmol}/10\ \text{min}$ per mg of protein. Means $\pm$ S.E.M. of five independent preparations are shown.

RESULTS AND DISCUSSION

Effect of Ba^{2+}

Fig. 1(a) shows the time course of $^{86}\text{Rb}^+$ uptake into basolateral-membrane vesicles from pars recta loaded with KCl ($55\ \text{mM}$) in the absence and presence of Ba^{2+} . In the absence of Ba^{2+} the uptake of the radioisotope reached a peak after 10 – $15\ \text{min}$, with a maximum uptake of about $205\ \text{pmol}/\text{mg}$ of protein. The presence of $10\ \text{mM}$ - Ba^{2+} decreased the $^{86}\text{Rb}^+$ uptake substantially. Fig. 1(b) summarizes the effect of increasing concentrations of Ba^{2+} on the $^{86}\text{Rb}^+$ uptake, and shows that the accumulation of radioisotope is decreased to only 40% of the control by a concentration of $1\ \text{mM}$ - Ba^{2+} in the medium. Addition of $100\ \text{mM}$ -tetraethylammonium did not change the radioisotope uptake significantly (result not shown).

Electrical nature of $^{86}\text{Rb}^+$ uptake

Table 1 summarizes experiments in which basolateral-membrane vesicles from pars recta were loaded with $55\ \text{mM}$ -KCl,

Table 1. $^{86}\text{Rb}^+$ uptake into basolateral-membrane vesicles from pars recta

The vesicles were loaded with $55\ \text{mM}$ -KCl, $27.5\ \text{mM}$ - K_2SO_4 or $27.5\ \text{mM}$ - Li_2SO_4 and incubated in the absence or presence of $10\ \text{mM}$ - BaCl_2 . The Li^+ ionophore was added to a final concentration of $10\ \mu\text{M}$. The uptakes were measured for $10\ \text{min}$. Control (100%) uptake was $195 \pm 12\ \text{pmol}$ of $^{86}\text{Rb}^+$ /10 min per mg of protein. Results are means $\pm$ S.E.M. ($n = 5$).

Intravesicular composition	Added to incubation medium	Relative $^{86}\text{Rb}^+$ uptake (%)
KCl		100
KCl	BaCl_2	32 ± 7
K_2SO_4		175 ± 35
Li_2SO_4		145 ± 15
Li_2SO_4	Li^+ ionophore	220 ± 29

$27.5\ \text{mM}$ - K_2SO_4 or $27.5\ \text{mM}$ - Li_2SO_4 , and the uptake of $^{86}\text{Rb}^+$ was measured. It is obvious from Table 1 that the rate of uptake into K_2SO_4 - or Li_2SO_4 -loaded vesicles is much higher than into KCl-loaded vesicles, and considerably higher into Li_2SO_4 -loaded vesicles pretreated with Li^+ ionophore. This agrees with the observation that the passive permeability of vesicle membranes is lower for sulphate than for chloride (Schneider & Sacktor, 1980). Since the membrane potential depends on the relative permeabilities and concentrations of all ions present, the rate of $^{86}\text{Rb}^+$ uptake should be higher in sulphate- than in chloride-loaded vesicles, and still higher in Li^+ -ionophore-pretreated and Li_2SO_4 -loaded vesicles. The uptake was enhanced by the factor 1.5 in the presence of the ionophore. These results demonstrate that the $^{86}\text{Rb}^+$ flux is driven by an electrical diffusion potential.

Ion selectivity

Fig. 2 and Table 2 summarize the information on the univalent-cation selectivity of the vesicles containing the K^+ channel. The selectivity was tested by loading vesicles with different cations

Fig. 2. $^{86}\text{Rb}^+$ uptake as a function of external cations

Basolateral-membrane vesicles from pars recta were loaded with $55\ \text{mM}$ -KCl, and the alkali chloride salts were added together with tracer $^{86}\text{Rb}^+$ to the incubation medium to a final concentration of $2\ \text{mM}$ (▨) or $10\ \text{mM}$ (▩). Maximum uptake (control) was $204 \pm 14\ \text{pmol}/10\ \text{min}$ per mg of protein.

Table 2. $^{86}\text{Rb}^+$ uptake as a function of internal cations

Basolateral-membrane vesicles from pars recta were loaded with 55 mM-KCl or 55 mM-NaCl, and uptakes were measured in the absence or presence of 1 mM- BaCl_2 , or loaded with 55 mM-RbCl, 55 mM-LiCl or 55 mM-choline chloride. The experimental protocol was as described in the legend to Fig. 1. The uptakes were measured for 10 min. Control (100%) uptake was 204 ± 14 pmol of $^{86}\text{Rb}^+$ /10 min per mg of protein. Results are means $\pm$ S.E.M. ($n = 5$).

Intravesicular composition	Added to incubation medium	Relative $^{86}\text{Rb}^+$ uptake (%)
KCl		100
KCl	BaCl_2	42 ± 9
RbCl		106 ± 6
LiCl		88 ± 9
Choline chloride		80 ± 22
NaCl		98 ± 16
NaCl	BaCl_2	36 ± 9

and examining the ability of those cations to sustain $^{86}\text{Rb}^+$ accumulation (Table 2). A high Rb^+ uptake should reflect a high permeability of the loaded cation as a result of a high electrical driving force. However, as shown in Table 2, no large differences were found for the various cations. Thus the method did not provide any ranking order of relative permeability of the cations. These findings were further tested in another way. The vesicles were loaded with 55 mM-KCl and the effect of adding alkali chloride to a final concentration of 2 or 10 mM together with $^{86}\text{Rb}^+$ was investigated. The result is shown in Fig. 2. The uptakes in the presence of 10 mM-KCl, 10 mM-RbCl, 10 mM-LiCl or 10 mM-NaCl decreased the $^{86}\text{Rb}^+$ uptake to a comparable extent, 30–35 % of the control (▨ bars). Choline chloride affected the $^{86}\text{Rb}^+$ uptake somewhat less. Similarly, there was no significant difference in the uptakes of $^{86}\text{Rb}^+$ when 2 mM of the same salts was added to the incubation medium together with $^{86}\text{Rb}^+$ (▩ bars). The most direct interpretation of these data is that addition of permeant cations to the outside medium depolarized the electrical gradient established across the channels and decreased the driving force for intravesicular accumulation of $^{86}\text{Rb}^+$. The depolarizing effect, and therefore the permeability, is approximately the same for K^+ , Rb^+ , Li^+ and Na^+ . The present findings are in contrast with what was found in basolateral-membrane vesicles isolated from pars convoluta. In this vesicle preparation similar experiments demonstrated the presence of Ba^{2+} -sensitive K^+ -selective channels (Jacobsen *et al.*, 1990b). In this connection, it is noteworthy that Gögelein & Greger (1986) in rabbit late proximal tubule (pars recta), using the patch-clamp technique, demonstrated non-selective channels which were equally permeable for Na^+ and K^+ . In addition, the same authors have, also by the patch-clamp technique, characterized a single K^+ channel present in basolateral membranes from the same region of the nephron (Gögelein & Greger, 1987). Our results indicate that we are operating with a non-selective channel in our basolateral-membrane vesicle preparation.

Inhibition of $^{86}\text{Rb}^+$ uptake by intravesicular Ca^{2+}

The inhibitory effect of intravesicular Ca^{2+} on the $^{86}\text{Rb}^+$ fluxes into luminal-membrane vesicles from pars recta of rabbit proximal tubule has been described previously (Jacobsen *et al.*, 1989a). 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 chosen concentration of free Ca^{2+} was achieved by incubation in a Ca^{2+} /EGTA buffer before adding tracer $^{86}\text{Rb}^+$

Fig. 3. Effect of Ca^{2+} on the $^{86}\text{Rb}^+$ uptake

Basolateral-membrane vesicles from pars recta were depleted of 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 $^{86}\text{Rb}^+$. Maximum uptake (control) was 194 ± 10 pmol/10 min per mg of protein.

(Pershadsingh & McDonald, 1980; Jacobsen *et al.*, 1990a). The same procedure was used in the present study. It appears from Fig. 3 that Ca^{2+} has a great impact on the $^{86}\text{Rb}^+$ influx. At 10 nM free Ca^{2+} the uptake is approx. 50 %, at 100 nM free Ca^{2+} it is approx. 40 % and at 10 μM - Ca^{2+} the uptake is below 20 % of the maximum uptake of $^{86}\text{Rb}^+$. The influence of Ca^{2+} on K^+ channels in basolateral membranes from distinct segments of the nephron has been investigated by the patch-clamp technique (Gögelein & Greger, 1987; Kawahara *et al.*, 1987; Sackin & Palmer, 1987; Parent *et al.*, 1988). By this technique, and in contrast with the present findings, basolateral K^+ channels in rabbit proximal straight tubule (pars recta) were found not to be directly dependent on Ca^{2+} ions (Gögelein & Greger, 1987). The same applied to K^+ channels in the basolateral membrane of *Necturus* proximal tubule, which were not affected by cytosolic-side Ca^{2+} at a concentration of 3 mM (Sackin & Palmer, 1987). However, Parent *et al.* (1988) observed a run-down in K^+ -channel activity of a K^+ -selective channel in the basolateral membrane of rabbit proximal convoluted tubule under any Ca^{2+} concentration. Our result clearly illustrates a pronounced effect of intravesicular Ca^{2+} on the $^{86}\text{Rb}^+$ uptake.

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