Potassium channels in the luminal membrane of rabbit proximal straight tubule

Evidence from vesicle studies

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The characteristics of $^{86}\text{Rb}^+$ fluxes through K^+ channels in luminal-membrane vesicles isolated from the pars recta of rabbit proximal tubule were studied. In KCl -loaded vesicles from the pars recta, transient accumulation of $^{86}\text{Rb}^+$ is observed which is modestly inhibited by BaCl_2 and blocked by CdCl_2 . The isotope accumulation is driven by an electrical diffusion potential, as shown in experiments using either these membrane vesicles loaded with different anions, or an outwardly directed Li^+ gradient with a Li^+ ionophore. The vesicles containing the channel show a cation selectivity with the order $\text{K}^+ > \text{Rb}^+ > \text{choline}^+ \geq \text{Li}^+ > \text{Na}^+$. The CdCl_2 -sensitive $^{86}\text{Rb}^+$ flux is dependent on intravesicular Ca^{2+} . Increasing concentrations of Ca^{2+} gradually decreased the $^{86}\text{Rb}^+$ uptake and at $1\ \mu\text{M}$ - Ca^{2+} the CdCl_2 -sensitive isotope flux is nearly abolished.

INTRODUCTION

The plasma vesicles of epithelial cells contain distinct luminal and basolateral domains, the series arrangements of which allow vectorial transport of solutes across the cell. The higher concentration of K^+ ions in the cell relative to the extracellular space is maintained by an ATP-driven Na^+/K^+ pump located in the basolateral membrane (for a review see Skou, 1965). The exit of K^+ from cells, on the other hand, is mediated by K^+ channels placed both in luminal and basolateral membranes. In recent years, attempts have been made to study the characteristics of K^+ -selective ion channels in membrane preparations of various 'tight' epithelia by the use of patch-clamp technique (for a review see Wills & Zweifach, 1987). However, progress in understanding epithelial K^+ channels has been hindered by a number of technical difficulties. Unlike the luminal-membrane Na^+ -selective channel, no specific blocker such as amiloride has been available for the epithelial K^+ channel. Secondly, there are many types of epithelial K^+ channels (Rae, 1985), and these types respond differently to various blocking agents (Van Driessche & Zeiske, 1985). In the case of leaky epithelia (i.e. kidney proximal tubule, intestine), very little information is available concerning K^+ -channel characteristics such as ion selectivity, competitive blocking, conformational states and regulatory mechanisms.

By the use of highly purified luminal-membrane vesicles from the pars recta (straight part) and pars convoluta (convoluted part) of the renal tubule, we have recently been able to demonstrate the existence of an amiloride-sensitive Na^+ channel in the straight segment of rabbit kidney proximal tubule (Jacobsen *et al.*, 1988). The main purpose of this series of studies is to investigate systematically the properties of K^+ -selective channels in luminal and basolateral membrane vesicles isolated from the two above-mentioned distinct regions of the nephron,

as an essential first step towards purification of the protein(s) involved in the transport process. In this article, we characterize a novel Ca^{2+} -regulated K^+ channel located in the pars recta of rabbit proximal tubule.

EXPERIMENTAL

Preparation of luminal-membrane vesicles

Luminal-membrane vesicles were isolated from the pars recta ('outer medulla') of the proximal tubule of rabbit kidney according to the protocol described previously (Sheikh & Møller, 1987). In all experiments $10\ \mu\text{M}$ of the Ca^{2+} ionophore A23187 and $3.75\ \text{mM}$ -EGTA were included in the washing medium of the vesicles to remove traces of intravesicular Ca^{2+} (Burnham *et al.*, 1986; Jacobsen *et al.*, 1988). The purity of the membrane vesicle preparations was examined by electron microscopy (Kragh-Hansen *et al.*, 1985) and by measuring specific activities of various enzyme markers as previously described (Sheikh *et al.*, 1982). The amount of protein was determined as described by Lowry *et al.* (1951), and as modified by Peterson (1977), with bovine serum albumin as a standard. All solutions used in this study were sterilized before use.

Assay of Rb^+ transport

Influx of $^{86}\text{Rb}^+$ was measured in K^+ ($55\ \text{mM}$)-loaded vesicles, analogous to flux-measurement procedures reported previously (Garty *et al.*, 1983; Jacobsen *et al.*, 1988). Dowex AG50W-X8 columns were prepared in $1\ \text{ml}$ tuberculin syringes. The resin was washed with $1\ \text{M}$ -Tris to convert it to the Tris form, followed by five to six volumes of $298\ \text{mM}$ -sucrose/ $3.75\ \text{mM}$ -EGTA/ $27\ \text{mM}$ -Hepes/Tris, pH 7.4, containing bovine serum albumin ($1\ \text{mg/ml}$). In the standard loading procedure, vesicles, $500\ \mu\text{l}$ (approx. $10\ \text{mg/ml}$), were suspended in a buffer of $185\ \text{mM}$ -sucrose, $3.75\ \text{mM}$ -EGTA, $1\ \text{mM}$ -ouabain, $55\ \text{mM}$ -KCl (or $55\ \text{mM}$ -RbCl, $-\text{NaCl}$, $-\text{choline chloride}$ or $27.5\ \text{mM}$ -

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Na_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 10 μM of the Li^+ ionophore *NN'*-diheptyl-*NN'*-5,5-tetramethyl-3,7-dioxanonanedi-*amides* (purchased from Fluka, Buchs, Switzerland). The vesicles were collected 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 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 , CdCl_2 and amiloride were added to final concentrations of 10 mM, 5 mM and 350 μM , respectively. In another set of experiments the inhibitory effect of Cd^{2+} was investigated with increasing concentrations of CdCl_2 (0.1–5 mM). The experiment was initiated by adding $^{86}\text{Rb}^+$ (210 kBq; 25 nmol) to the incubation medium. Samples of 140 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 columns by centrifugation. The eluates were diluted to 500 μl and used 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 1.5–4.85 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 according to Pershadsingh & McDonald (1980).

RESULTS AND DISCUSSION

Effect of external Ba^{2+} and Cd^{2+}

Fig. 1 shows the time course of $^{86}\text{Rb}^+$ uptake into luminal-membrane vesicles from pars recta loaded with KCl (55 mM) in the absence or presence of Ba^{2+} or Cd^{2+} ions (for a detailed description of the principles involved in measuring isotope fluxes through ion channels see Garty *et al.*, 1983). In the absence of bivalent ions, the uptake of the isotope against the opposing K^+ concentration gradient rose to a peak value at about 10 min and then started to decline (Fig. 1a, $\bigcirc$). In the absence of 10 mM- Ba^{2+} , $^{86}\text{Rb}^+$ uptake is modestly reduced (approx. 20% of control) as shown in Fig. 1 (a, $\bullet$). Addition of tetraethylammonium (10 mM) reduced the uptake of isotope to the same value (not shown). However, application of 5 mM- Cd^{2+} , which has been shown to block conductance in human T cells (Bregestovski *et al.*, 1986), drastically reduced the uptake of $^{86}\text{Rb}^+$ in our vesicle preparations (Fig. 1a, $\blacksquare$). Fig. 1(b) summarizes the effect of various concentrations of CdCl_2 (ranging from 0.1 to 5 mM) on the uptake of $^{86}\text{Rb}^+$ in vesicles from the pars recta. It is apparent from the Figure that the accumulation of isotope gradually decreases with an increasing medium concentration of Cd^{2+} . In this connection, it is of interest to note that Wills and co-workers (Wills *et al.*, 1982; Wills & Zweifach, 1987) have identified an apical-membrane K^+ conductance in the mammalian colon, which is large enough to support net K^+ secretion by this epithelium. So far, further characterization of apical-membrane K^+ channels in the mammalian colon has been impeded by a lack of effective blockers for this channel. The commonly used blocker, Ba^{2+} , has no significant effect on K^+ -current

Fig. 1. Time course of $^{86}\text{Rb}^+$ uptake into luminal-membrane vesicles from the pars recta rabbit proximal tubules

(a) Vesicles (1 ml; protein concn. 2.6 mg/ml), intracellular K^+ concentration, 55 mM, extracellular K^+ concentration 0 mM, were incubated at room temperature with $^{86}\text{Rb}^+$ (17 μM) without inhibitor ($\bigcirc$), in presence of 10 mM- BaCl_2 ($\bullet$), or in presence of 5 mM- CdCl_2 ($\blacksquare$). (b) The effect of 0.1–5 mM- Cd^{2+} in the incubation medium on the uptake of $^{86}\text{Rb}^+$. Control, 100% uptake, is without Cd^{2+} in the incubation medium. Means $\pm$ S.E.M. of four independent preparation are shown.

noise or basal K⁺ secretion in this epithelium, while tetraethylammonium and caesium ions only partially block the channel and K⁺ fluxes (Wills *et al.*, 1982; Wills & Zweifach, 1987). Weak interactions of blocker with the apical-membrane K⁺ channel have also been reported for the gastric mucosa (Zeiske *et al.*, 1983).

To examine whether the Cd²⁺-insensitive ⁸⁶Rb⁺ uptake may be due to passive Rb⁺/K⁺ exchange through the Na⁺/K⁺-exchange pump (Karlsh & Stein, 1982; Kenney & Kaplan, 1985), a series of experiments were performed in which the effect of ouabain on the uptake of isotope was measured. The results of these experiments showed that addition of 1 mM-ouabain slightly reduced the Cd²⁺-insensitive uptake (approx. 10%). In the experiments described below, 1 mM-ouabain has been included in the incubation medium. We have also examined the effect of amiloride (350 μM) on the uptake of ⁸⁶Rb⁺ in membrane vesicles. No significant inhibitory effect of this diuretic on the uptake of isotope was observed, suggesting that ⁸⁶Rb⁺ influx in vesicles from the pars recta predominantly occurs through K⁺ channels.

Electrical nature of ⁸⁶Rb⁺ uptake

Data presented in Table 1 provide direct evidence that the ⁸⁶Rb⁺ accumulation is driven by an electrical potential. In these experiments, the luminal-membrane vesicles from the pars recta were loaded with 55 mM-KCl, 27.5 mM-K₂SO₄ or 55 mM-KSCN and the uptake of ⁸⁶Rb⁺ was studied in the absence and presence of Ba²⁺ or Cd²⁺. It is apparent from the Table that the rate of uptake of isotope was highest for sulphate, intermediate with chloride medium and lowest with thiocyanate. The results can be explained as follows. It is known that the passive permeability of the vesicle membrane for sulphate is lower than that for chloride and the permeability for thiocyanate is higher than that for chloride (Frömter *et al.*, 1971; Schneider & Sacktor, 1980). The cation diffusion potential should therefore be progressively short-circuited by the anion of increasing permeability (i.e. SO₄²⁻ < Cl⁻ < SCN⁻), and hence the rate of the ⁸⁶Rb⁺ uptake should follow the observed order SCN⁻ < Cl⁻ < SO₄²⁻.

The above-mentioned theory was further tested by

Table 1. ⁸⁶Rb⁺ uptake into luminal-membrane vesicles from the pars recta

The vesicles were loaded with 55 mM-KCl, 27.5 mM-K₂SO₄, 27.5 mM-Li₂SO₄ or 55 mM KSCN and incubated in the absence and presence of 10 mM-BaCl₂ or 5 mM-CdCl₂. The Li⁺ ionophore was added to a final concentration of 10 μM. The uptakes were measured for 5 min. Results are means ± S.E.M. (n = 4).

Intravesicular composition	Addition to incubation medium	Relative ⁸⁶ Rb ⁺ uptake (%)
KCl		100
	BaCl ₂	76 ± 5.5
	CdCl ₂	26 ± 4.3
K ₂ SO ₄		123 ± 7.6
KSCN		78 ± 5.5
Li ₂ SO ₄		155 ± 5.0
	Li ionophore	222 ± 18.0

examining the effect of Li⁺ ionophore on the uptake of ⁸⁶Rb⁺ in Li₂SO₄-loaded vesicles. In these experiments, the luminal-membrane vesicles from the pars recta were loaded with 27.5 mM-Li₂SO₄ for 90 min at 0 °C, and these vesicles were then passed through a Tris-equilibrated column and portions were incubated for 1 min with the ionophore or with dimethyl sulphoxide (0.5%, w/v) and ⁸⁶Rb⁺ uptake was measured. Furthermore, Table 1 shows that the induced Li⁺ diffusion potential enhanced the influx of isotope in these vesicle preparations. The results established that the Cd²⁺-insensitive ⁸⁶Rb⁺ uptake is driven by an electrical diffusion potential.

Ion selectivity

Fig. 2 summarizes information on the univalent cation selectivity of the vesicles containing the K⁺ channel. The selectivity to cations was tested by loading vesicles with different cations and examining the ability of an opposing gradient of various cations to sustain ⁸⁶Rb⁺ accumulation. The observed ranking order K⁺ > Rb⁺ > choline⁺ ≥ Li⁺ > Na⁺ is a reflection of the decreasing electrical potential driving ⁸⁶Rb⁺ accumulation and, therefore, of the relative permeability of the membrane vesicles of interest to these cations. The result could mean that the K⁺ channels themselves show this order of selectivities, but it is more probable that the K⁺ channel shows a higher selectivity to K⁺ than, e.g. Na⁺.

Fig. 2. Time course of ⁸⁶Rb⁺ uptake into luminal-membrane vesicles from the pars recta

The vesicles were loaded with 55 mM-KCl (○), 55 mM-RbCl (△), 55 mM-choline chloride (□), 55 mM-LiCl (◇) or 55 mM-NaCl (▽). The experimental protocol was as described in the legend to Fig. 1. Results are mean values from two independent preparations.

and, in addition, the membrane vesicles are somewhat selective for all cations compared with anions.

Inhibition of K⁺-channel activity by intravesicular Ca²⁺

In recent years, a number of K⁺ channels have been shown to be activated by intracellular Ca²⁺ (Wills & Zweifach, 1987). Ca²⁺-activated channels have been identified in many cell types, including erythrocytes, neurons, muscles, endocrine and exocrine glands (Petersen & Maruyama, 1984) and, more recently, in luminal-membrane vesicles isolated from mammalian renal thick ascending limb of the loop of Henle (Burnham *et al.*, 1985, 1986; Klaerke *et al.*, 1987). The use of the patch-clamp technique has allowed workers to distinguish three different categories of Ca²⁺-activated K⁺ channels, two of which are K⁺ selective and voltage sensitive, but differ in the single-channel conductance and a third class which appears to be voltage insensitive and non-selective to cations.

It has previously been shown that incubation with a high concentration of EGTA and Ca²⁺ ionophore A23187 in combination is required for depletion of Ca²⁺ and reduction of K⁺-channel activity in luminal-membrane vesicles isolated from the thick ascending limb of loop of Henle of rabbit kidney (Burnham *et al.*, 1985, 1986). Exactly the same procedures have been used in this study to remove intravesicular Ca²⁺. Fig. 3 shows the effect of increasing intravesicular Ca²⁺ concentrations on the uptake of K⁺ by luminal-membrane vesicles from the pars recta. It appears from Fig. 3 that at very low intravesicular concentrations of Ca²⁺ the influx of ⁸⁶Rb⁺ is not significantly different, but at a relatively high Ca²⁺ concentration (100 nM), the Cd²⁺-sensitive flux of isotope is reduced to approx. 50% of the maximal uptake of ⁸⁶Rb⁺. At 1 μ M intravesicular Ca²⁺, the Cd²⁺-sensitive ⁸⁶Rb⁺ flux is nearly abolished. This is in contrast to other systems in which intracellular Ca²⁺ activates K⁺ channels. Burnham *et al.* (1985, 1986) and Klaerke *et al.* (1987) have reported that the K⁺ channel present in the luminal-membrane vesicles isolated from the ascending limb of loop of Henle of rabbit kidney was activated by intravesicular Ca²⁺, and this K⁺ channel was shown to be completely blocked by 5 mM-Ba²⁺. Incidentally, the above-mentioned findings ruled out the possibility of appreciable contamination of pars recta vesicles with the membrane fragments from the ascending limb of loop of Henle. The inset in Fig. 3 shows the effect of various concentrations of Mg²⁺ on the uptake of ⁸⁶Rb⁺. It is clear from the Figure that Mg²⁺ ions do not affect the uptake of isotope indicating that the uptake of isotope is specifically regulated by Ca²⁺ ions.

The results presented above on the properties of the K⁺ channel in the luminal-membrane vesicles of the pars recta have some features in common with the characteristics of the K⁺ channel found in T lymphocytes (Bregestovski *et al.*, 1986). Using the patch-clamp technique, Bregestovski *et al.* (1986) showed an unexpected relationship between the level of intracellular Ca²⁺ in human lymphocytes and the amplitude of a voltage-dependent current: the elevation of the intracellular Ca²⁺ concentration decreased the K⁺ conductance. Furthermore, application of 5 mM-Cd²⁺ completely blocked the K⁺-channel activity in the human T cell, similar to the observations reported here. Those authors suggested that the level of intracellular Ca²⁺ regulates the effective number of K⁺ channels capable of being activated.

Fig. 3. Effect of Ca²⁺ on the ⁸⁶Rb⁺ uptake into luminal-membrane vesicles from the pars recta

The vesicles were depleted of Ca²⁺ by treatment with 3.75 mM-EGTA and 10 μ M-Ca²⁺ ionophore A23187 for 20 min at 20 °C 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 1.5–4.75 mM-CaCl₂ to give the desired concentration of free Ca²⁺ before adding tracer ⁸⁶Rb⁺. As controls, vesicles loaded with KCl (55 mM) and incubated in the absence (○) and presence of 10 mM-BaCl₂ (●) or 5 mM-CdCl₂ (■) are shown. The inset shows the uptake in the presence of various concentrations of MgCl₂ without and with 1 μ M-CaCl₂ (hatched areas). Results are mean values from two independent preparations.

Whether intracellular Ca²⁺ regulates the K⁺ channel of the renal proximal straight tubule according to their proposal remains to be investigated

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