Volume 243, number 2, 173–176 FEB 06719 January 1989

Ba^{2+}-sensitive K^+ channels in luminal-membrane vesicles from pars convoluta of rabbit proximal tubule

Christian Jacobsen, Hans Røigaard-Petersen and M.I. Sheikh

Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

Received 16 November 1988

This paper describes properties of $^{86}Rb^+$ fluxes through a novel K^+ channel in luminal-membrane vesicles isolated from pars convoluta of rabbit proximal tubule. The uptake of $^{86}Rb^+$ into potassium salt loaded vesicles was specifically inhibited by Ba^{2+}. The isotope accumulation is driven by an electrical diffusion potential as shown in experiments using these membrane vesicles loaded with anions of different membrane permeability and was as follows: gluconate $> SO_4^{2-} > Cl^-$. Furthermore, the vesicles containing the channels show a cation selectivity with the order $K^+ > Rb^+ > Li^+ > Na^+ =$ choline$^+$.

K^+ channel; Ba^{2+} sensitivity; Membrane vesicle; (Pars convoluta)

1. INTRODUCTION

Ionic channels constitute substantial routes for the movement of ions across epithelial cell membranes. The luminal membrane is essentially conductive for Na^+, while the basolateral membrane is generally conductive for K^+. However, in certain epithelia such as mammalian colon [1–3] and proximal tubule [4–7], there is considerable evidence in the literature for the existence of K^+ channels both in luminal and basolateral membranes. By the use of highly purified luminal-membrane vesicles from two distinct segments of nephron we have recently been able to demonstrate the presence of an amiloride-sensitive Na^+ channel in pars recta of rabbit proximal tubule [8]. Using the same experimental procedure as described in [8,9], we now describe some characteristics of a novel Ba^{2+}-sensitive K^+ channel in luminal-membrane vesicles isolated from pars convoluta of rabbit proximal tubule.

Correspondence address: M.I. Sheikh, Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

2. MATERIALS AND METHODS

2.1. *Preparation of luminal-membrane vesicles*

Luminal-membrane vesicles were isolated from pars convoluta (outer cortex) of the proximal tubule of rabbit kidney according to [10] and the method is only briefly described here. Outer cortical tissue was obtained by taking slices < 0.3 mm thick from the surface of the kidney containing pars convoluta. The tissue was homogenized and vesicles were prepared by differential centrifugation and by Mg^{2+} precipitation analogous with Ca^{2+} precipitation as described in [10]. The final pellet was washed for 20 min by a solution consisting of 3.75 mM EDTA, 298 mM sucrose and 27 mM Hepes/Tris buffer, pH 7.4, and resuspended in the same medium minus EDTA. The purity of the membrane vesicle preparations was examined by electron microscopy [11] and by measuring specific activities of various enzyme markers as in [12]. The amount of protein was determined as described by Lowry et al. [13] and as modified by Peterson [14] and with serum albumin as a standard. All solutions used in this study were sterilized before use.

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 [8,9]. Dowex AG50W-X8 columns were prepared in 1 ml tuberculin syringes. The resin was washed with 1 M Tris to bring it into the Tris form, followed by 5–6 vols of 298 mM sucrose, 27 mM Hepes/Tris, pH 7.4, containing 1 mg/ml bovine serum albumin. In the standard loading procedure, vesicles, 500 μl (approx. 10 mg/ml), were suspended in a buffer of 185 mM sucrose, 3.75 mM EDTA, 1 mM ouabain, 55 mM KCl (or

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

55 mM RbCl, NaCl, LiCl, choline chloride, K-gluconate or 27.5 mM K_2SO_4), 27 mM Hepes/Tris, pH 7.4, for 1.5 h on ice. 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 of 300 μl were applied on separate columns. The individual eluates were diluted to 1.0 ml with 298 mM sucrose, 27 mM Hepes/Tris, pH 7.4, buffer, and 75 μl were withdrawn for protein determination. In references $BaCl_2$ and amiloride were added in final concentrations of 10 mM and 350 μM, respectively. The experiment was initiated by adding $^{86}Rb^+$ (210 kBq; 25 nmol) to the incubation medium. Samples of 140 μl were taken out at various time intervals (1, 3, 5, 7, 10, 15 and 60 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.

3. RESULTS AND DISCUSSION

3.1. *Effects of external Ba^{2+} and ouabain*

Fig.1 presents the intravesicular $^{86}Rb^+$ content as a function of time in the absence and presence of 10 mM Ba^{2+} in KCl (55 mM) loaded luminal-membrane vesicles from pars convoluta. With or without external Ba^+ the vesicles continued to accumulate $^{86}Rb^+$ throughout the 15 min of measurements. It is seen that the external Ba^{2+} reduced the magnitude of the $^{86}Rb^+$ uptake throughout the entire period of measurement, suggesting that part of the influx proceeded through aqueous channels. Barium is widely used as a blocker of K^+ channels in epithelial cells (review [15]). It is likely that part of the Ba^{2+}-insensitive $^{86}Rb^+$ uptake reflects passive Rb^+/K^+ exchange through the Na^+,K^+-exchange pump [16,17]. In a separate experiment, addition of 1 mM ouabain slightly reduced (approx. 10%) the Ba^{2+}-insensitive uptake. In the present communication, we have been concerned specifically with transport through aqueous channels. Therefore, in order to reduce the level of the background Ba^{2+}-insensitive $^{86}Rb^+$ flux, 1 mM ouabain has been included in the incubation medium in all of the experiments described below. We have also examined the effect of amiloride (350 μM) on the uptake of $^{86}Rb^+$. No significant inhibitory effect of this diuretic on the uptake of isotope was observed (not shown), suggesting that influx of $^{86}Rb^+$ occurred via a K^+ channel. Incidentally, these findings rule out the possibility of appreciable contamination of pars convoluta vesicles with the membrane fragments from pars recta, in which Na^+ channels are shown to be present [8].

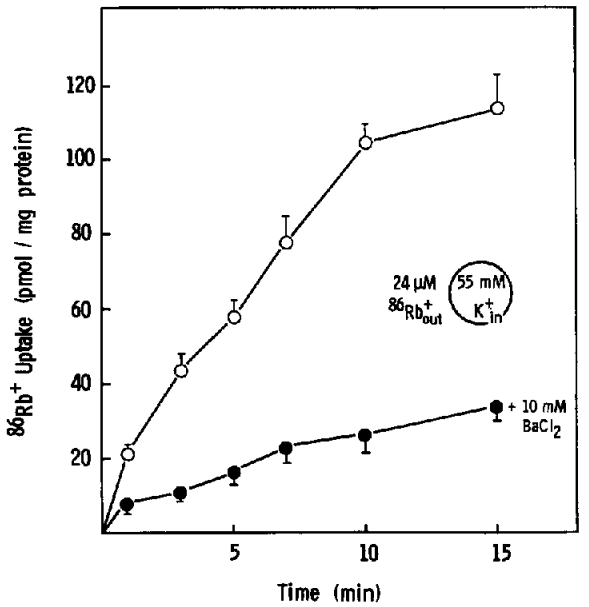

Fig.1. Time course of $^{86}Rb^+$ uptake into luminal-membrane vesicles from pars convoluta of rabbit proximal tubules. One ml of vesicles (protein conc. 5 mg/ml), K^+_{in} = 55 mM, K^+_{out} = 0 mM, was incubated at room temperature with $^{86}Rb^+$ (24 μM) in the absence (curve 1, ○), and presence of 10 mM $BaCl_2$ (curve 2, ●). Means ± SE of four independent preparations are shown.

3.2. *Cation selectivity*

Whether or not the $^{86}Rb^+$ transport is conductive is further explored by examining the effect of loading vesicle with various alkali cations on the influx of $^{86}Rb^+$. In these experiments, the vesicles were loaded with different cations and the ability of opposing gradients of these cations to sustain Ba^{2+}-sensitive $^{86}Rb^+$ accumulation was examined. Fig.2A shows that all the cations tested in this paper reduced the uptake of $^{86}Rb^+$ to various extents as compared to the influx of $^{86}Rb^+$ by K^+-loaded vesicles (control). Thus, these measurements indicate that the radioactive tracer movement in the presence of ouabain proceeds largely through a conductive pathway. The observed ranking order $K^+ > Rb^+ > Li^+ > Na^+ = choline^+$ is a reflection of the decreasing permeability of the membrane vesicle of interest to these cations. The results could mean that the K^+ channels themselves show this order of selectivities, but it is more probable that K^+ channels show a higher selectivity to K^+ than to Na^+, and in addition the membrane

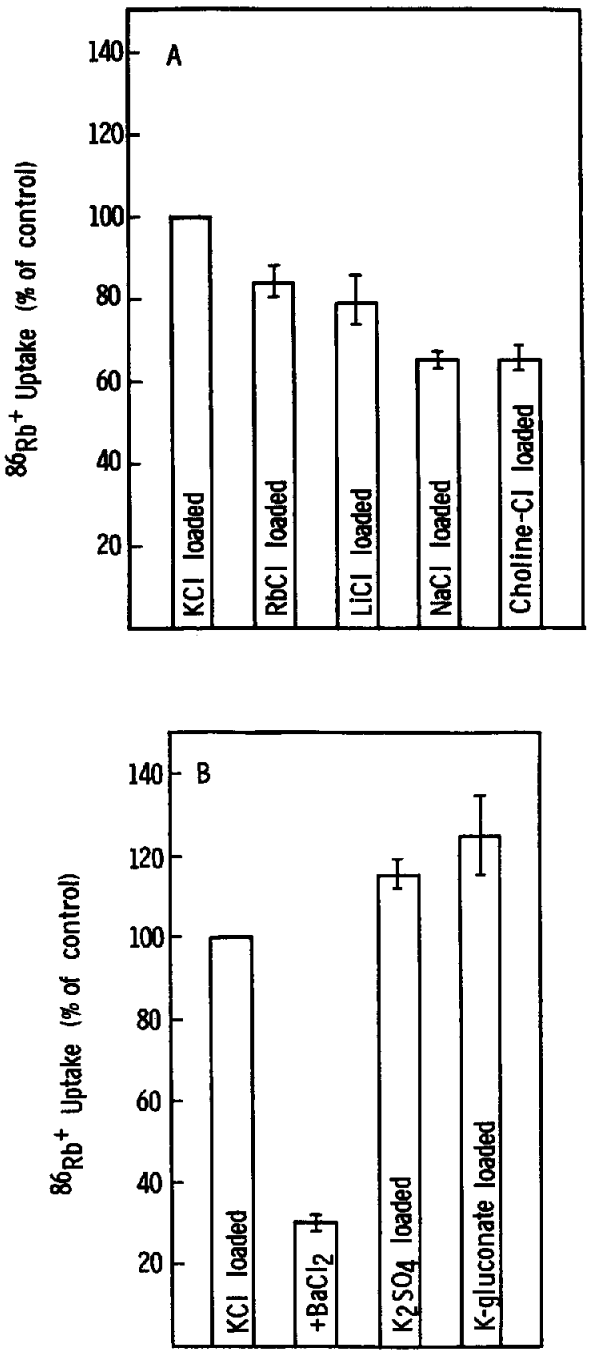

Fig.2. Time course of $^{86}Rb^+$ uptake into luminal-membrane vesicles from pars convoluta. (A) Vesicles loaded with either 55 mM KCl, 55 mM RbCl, 55 mM LiCl, 55 mM NaCl or 55 mM choline chloride. (B) Vesicles loaded with either 55 mM KCl, 27.5 mM K_2SO_4 or 55 mM K-gluconate and measured in absence and presence of 10 mM $BaCl_2$. The experimental protocol was as described in legend to fig.1.

vesicles are somewhat selective for all cations compared to anions.

3.3. *Electrical nature*

Fig.2B provides direct evidence that the Ba^{2+}-inhibited $^{86}Rb^+$ accumulation is driven by an electrical potential. In these experiments the luminal-membrane vesicles from pars convoluta were loaded with either 55 mM KCl, 27.5 mM K_2SO_4 or 55 mM K^+-gluconate, and the uptake of $^{86}Rb^+$ was studied in the absence and presence of Ba^{2+}. It is apparent from fig.2B that the rate of isotope uptake was highest for gluconate, intermediate with sulfate medium and lowest with chloride. The results can be explained as follows. It is known that the passive permeability of the vesicle membrane for these anions is as follows: gluconate $< SO_4^{2-} < Cl^-$ [18,19]. The cation diffusion potential should therefore be progressively short-circuited by anions of increasing permeability and the rate of the $^{86}Rb^+$ uptake should follow the observed order gluconate $> SO_4^{2-} > Cl^-$.

In conclusion, the results presented in this communication revealed the presence of a novel Ba^{2+}-sensitive K^+ channel in luminal-membrane vesicles prepared from pars convoluta of rabbit proximal tubule. Furthermore, our preliminary observation indicates that the K^+ channel activity is regulated by Ca^{2+}. These experiments are in progress in our laboratory.

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 Lægevidenskabens Fremme, Kong Christian Den Tiendes Fond, Fogh-Nielsens Legat and NOVO Fond.

REFERENCES

[1] Wills, N.K., Zeiske, W. and Van Driessche, W. (1982) J. Membrane Biol. 69, 187–197.
[2] Wills, N.K., Alles, W.P., Sandler, G.I. and Binder, H.J. (1984) Am. J. Physiol. 247, 749–757.
[3] Dawson, D.C., Van Driessche, W. and Helman, S.I. (1985) Fed. Proc. 44, 1745.
[4] Gögelein, H. and Greger, R. (1984) Pflügers Arch. 401, 424–426.
[5] Parent, L., Cardinal, J. and Sauve, R. (1986) Biophys. J. 49, 159a.
[6] Gögelein, H. and Greger, R. (1987) Pflügers Arch. 410, 288–295.

[7] Parent, L., Cardinal, J. and Sauve, R. (1988) Am. J. Physiol. 254, F105–F113.
[8] Jacobsen, C., Røigaard-Petersen, H. and Sheikh, M.I. (1988) FEBS Lett. 236, 95–99.
[9] Garty, H., Rudy, B. and Karlish, J.D. (1983) J. Biol. Chem. 258, 13094–13099.
[10] Sheikh, M.I. and Møller, J.V. (1987) in: Biochemical Toxicology: A Practical Approach (Snell, K. and Mullock, B. eds) Ch.7, pp.153–182, IRL Press, Oxford, England.
[11] Kragh-Hansen, U., Røigaard-Petersen, H. and Sheikh, M.I. (1985) Am. J. Physiol. 249, F704–F712.
[12] Sheikh, M.I., Kragh-Hansen, U., Jørgensen, K.E. and Røigaard-Petersen, H. (1982) Biochem. J. 208, 377–382.
[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[14] Peterson, G.L. (1977) Anal. Biochem. 83, 346–356.
[15] Wills, N.K. and Zweifach, A. (1987) Biochim. Biophys. Acta 906, 1–31.
[16] Karlish, S.J.D. and Stein, W.D. (1982) J. Physiol. (London) 328, 295–316.
[17] Kenney, L.J. and Kaplan, J.H. (1985) in: The Sodium Pump (Glynn, I.H. and Ellory, J.C. eds) pp.535–539, The Company of Biologists Ltd, Cambridge.
[18] Frömter, E., Muller, C.W. and Wick, T. (1971) in: Electrophysiology of Epithelial Cells (Giebisch, G. ed.) pp.119–146, F.K. Schattauer, Stuttgart.
[19] Schneider, E.G. and Sacktor, B. (1980) J. Biol. Chem. 255, 7645–7649.