Potassium Channels of the Lamprey Erythrocyte Membrane Exhibit a High Selectivity to K^+ over Rb^+: a Comparative Study of ^{86}Rb and ^{41}K Transport

G P Gusev, D G Fleishman, V A Nikiforov, and A O Sherstobitov

Sechenov Institute of Evolutionary Physiology and Biochemistry
Russian Academy of Sciences
St Petersburg Russia

Abstract. In order to assess the specificity of potassium channels, we examined the K^+ transport pathways in the lamprey red blood cells using ^{86}Rb and stable isotope ^{41}K as tracers measured by the mass spectrometric method Upon replacing 4 mmol/l K^+ with 4 mmol/l Rb^+ in the incubation medium, the rate coefficient for unidirectional ^{86}Rb influx (K_{in}) was significantly reduced from $1\ 48 \pm 0\ 10\ h^{-1}$ to $0\ 82 \pm 0\ 05\ h^{-1}$ ($P < 0\ 001$) Addition of 1 mmol/l Ba^{2+} to the incubation medium significantly decreased ^{86}Rb influx in K^+- (by 53%) and Rb^+-media (by 26%) Thus, the reduction in the rate coefficient for ^{86}Rb influx in Rb^+-medium as compared to K^+-medium was mainly due to the Ba^{2+}-sensitive component ($0\ 23 \pm 0\ 04$ vs $0\ 78 \pm 0\ 10\ h^{-1}$, $P < 0\ 001$) The ouabain-sensitive component of ^{86}Rb influx was also higher in K^+-($K_{in} = 0\ 62 \pm 0\ 05\ h^{-1}$) than in Rb^+-medium ($K_{in} = 0\ 54 \pm 0\ 05\ h^{-1}$, $P < 0\ 05$) In the presence of 4 mmol/l ^{41}KCl, the average value of the rate coefficient for the total ^{41}K influx was $8\ 1 \pm 0\ 5\ h^{-1}$ The rate coefficient for ^{41}K influx was reduced to $0\ 82 \pm 0\ 13\ h^{-1}$ in the presence of 1 mmol/l Ba^{2+} Under the conditions of our assays, the rate of ^{86}Rb uptake via potassium channels of the lamprey red cells was only about 11% in K^+-medium and only about 3% in Rb^+-medium compared to ^{41}K influx These data clearly demonstrate a high selectivity of potassium channels for K^+ over Rb^+, as already reported for some potassium channels in a variety of cells and tissues

Key words: K^+ channels — Na^+, K^+-pump — K^+/Rb^+ selectivity — Lamprey erythrocytes

Correspondence to G P Gusev, Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, 194223, Thorez pr 44 St Petersburg, Russia Tel (812) 552-32-91, Fax (812) 552-30-12 E-mail gusev@ief spb su

Introduction

There has been a large number of studies that suggested the ability of Rb^+ to substitute satisfactorily for K^+ in the investigation of ion transport mechanisms in a variety of cells and tissues (Castronovo et al. 1968; Beauge and Ortiz 1970; Bernstein and Israel 1970; Cheval et al. 1991; Ikehara et al. 1993). Therefore, K^+ transport through cell membranes is often assessed by measuring ^{86}Rb because of the cost and the short half-life of potassium radionuclides. However, some potassium channels have long been known to be able to discriminate between these two ions (Adrian 1964; Hille 1973, Lattore and Miller 1983; Gallacher et al 1984; Van Driessche and Zeiske 1985; Silver et al. 1994). This selectivity for K^+ over Rb^+ is a property shared by many of the numerous well-defined potassium channels, although the degree of selectivity differs among classes of the channels

The lamprey red blood cells are unique among erythrocytes of many other species studied so far. The lamprey erythrocyte membrane shows a low chloride permeability (Nikinmaa and Railo 1987; Gusev and Sherstobitov 1993). On the other hand, several recent studies (Kirk 1991; Gusev et al. 1992) have shown that the lamprey red cells contain potassium channels which can be inhibited by Ba^{2+}, quinine, tetraethylammonium and amiloride. The channels provide a major contribution to total K^+ influx in the red cells under physiological conditions, but their properties remain unexplored. In previous investigations the pathways of K^+ movement through the lamprey erythrocyte membrane have been examined using ^{86}Rb as a tracer. Kirk (1991) has observed that the transport of ^{86}Rb in the lamprey erythrocytes was definitely smaller than that of ^{43}K when the incubation medium contained 4 mmol/l K^+ However, in this study no detailed experiments have been done with replacing external K^+ with Rb^+ to ascertain the true K^+/Rb^+ selectivity for the potassium channels in the lamprey erythrocytes. Therefore, it is of interest to define more clearly the discrimination between K^+ and Rb^+ transport via the potassium channels in the lamprey erythrocyte membrane which is a convenient model for investigating ion transport mechanisms.

The present study was undertaken to examine the extent to which Rb^+ would substitute for K^+ in the transport pathways through the lamprey erythrocyte membrane. We combined two approaches to study K^+ and Rb^+ transfer across the cell membrane. First, the rates of Ba^{2+}- and ouabain-sensitive ^{86}Rb uptake were compared at the presence of 4 mmol/l KCl or 4 mmol/l RbCl in the media. Secondly, the rate of Ba^{2+}-sensitive influx of the stable isotope ^{41}K into the red cells was studied in media containing 4 mmol/l ^{41}KCl. The data presented in this paper indicate that the potassium channels are highly selective for K^+ over Rb^+. In contrast there is a relatively small discrimination ability (~20%) between K^+ and Rb^+ transport via the Na-K-pump.

Materials and Methods

Animals

Experiments were carried out on river lampreys (*Lampetra fluviatilis*) weighing 50–80 g. The animals were kept for 4 months (November–February) in aquaria with tap water at 2–4 °C under continuous aeration. The water in the aquaria was periodically replaced with fresh dechlorinated tap water.

Preparation of cells

Blood was drawn after rapid decapitation in a tube containing heparinized saline. Red cells were separated by centrifugation (2700× *g* for 5 min). After aspiration of saline and the buffy coat, the erythrocytes were washed three times in a standard saline containing 145 mmol/l NaCl and 10 mmol/l Tris-HCl (pH 7.4 at 20 °C). Cell suspensions in saline (hematocrit 30–40%) were stored at room temperature for no more than 10 min before experiments.

Measurement of ^{86}Rb and ^{41}K uptake

K^+ and Rb^+ influxes were measured using radioactive ^{86}Rb and stable isotope ^{41}K as tracers. All experiments were performed at room temperature (18–20 °C). Incubation media were prepared by adding to the standard saline of 10 mmol/l glucose and 140 mmol/l KCl, RbCl or ^{41}KCl solutions to a final concentration of 4 mmol/l. Flux media also contained 1 mmol/l $BaCl_2$ or 0.1 mmol/l ouabain as required. The cell suspension was added to the incubation media to a final hematocrit of 2–3%. Then, ^{86}Rb (0.01 mCi/ml) was added to the media containing KCl and RbCl at zero time. At various time intervals 1 ml aliquots of well mixed suspension were removed and injected into 10 ml ice-cold standard saline. After centrifugation (2700× *g* for 1 min at 4 °C) a small amount of the supernatant was taken for the determination of radioactivity. The red cells were washed twice with the same ice-cold saline. Cell pellets were lysed in distilled water, the radioactivity of the media and lysates was determined using a gamma-counter. As shown in this study ^{86}Rb is an inaccurate tracer for the measurement of K^+ transport through the lamprey erythrocyte membrane. Therefore, ^{86}Rb uptake was calculated as follows: $J_{uj} = A_{RBC}/A_M$ where A_{RBC} is the radioactivity of 1 ml packed cells and A_M is the radioactivity of 1 ml medium. As ^{86}Rb uptake was linear up to 60 min (Fig. 1), the rate coefficients for ^{86}Rb influx (h^{-1}) were calculated from 60 min ^{86}Rb uptake. At the same concentration of K^+ and Rb^+ in media, the rate coefficients reflect ion influxes (mmol/l cells/h).

In the experiments with ^{41}KCl, the $^{41}K/^{39}K$ isotope ratio was measured in washed erythrocytes using a mass spectrometer with the triple filament ion source (Fleishman et al. 1992). In brief, 20–30 μl of washed erythrocytes were lysed in 5 ml distilled water and about 3 μl of lysates was deposited on one evaporator of the ion

source. The isotope composition of endogenous (natural) potassium corresponds to a $^{41}K/^{39}K$ ratio of 0.072. The intracellular K^+ concentration in the washed red cells was determined using a Flapho-40 flame photometer (Jena, Germany). As could be shown in our experiments, the concentration of intracellular K^+ remained constant at any incubation period. The K^+ concentration, measured by flame photometry (C_K), is a sum of C_{41K} and C_{39K} or $C_K = C_{41K} + C_{41K}/r$, where r is the C_{41K}/C_{39K} ratio. Thus, the concentration of ^{41}K in the red cells can be calculated as follows: $C_{41K} = C_K \cdot r/(1 + r)$. At zero time the concentration of endogenous ^{41}K was equal to $C_K \cdot 0.072/(1 + 0.072)$. ^{41}K uptake by the lamprey erythrocytes was calculated as the difference between ^{41}K concentration at time t and the endogenous content of ^{41}K. The ^{41}K uptake was expressed as mmol per liter packed cells. The rate coefficient for ^{41}K uptake was calculated from the initial linear ^{41}K uptake divided by ^{41}K concentration in the medium and the incubation time.

Reagents

All chemicals used were of analytical grade. Ouabain was obtained from Sigma (St. Louis, MO, USA). Ouabain was dissolved in a flux medium to make a 10 mmol/l stock solution. ^{86}Rb and ^{41}KCl (96% ^{41}K, 4% ^{39}K) were obtained from ISOTOP (St. Petersburg, Russia).

Statistics

All results are given as the mean value ± standard error (S.E.). Statistical significance was determined by the Student's t-test for paired observations.

Results

Time course of ^{86}Rb uptake in K^+- and Rb^+-media

The first series of experiments was undertaken to compare ^{86}Rb uptake by the lamprey red cells incubated in media containing 4 mmol/l K^+ or 4 mmol/l Rb^+. As can be seen in Fig. 1*A*, the rate of ^{86}Rb uptake was significantly greater in K^+ medium than in Rb^+-medium. In both media the ^{86}Rb uptake by lamprey erythrocytes was approximately linear up to 60 min of incubation. The calculated rate coefficients for ^{86}Rb uptake were 1.66 ± 0.16 h^{-1} in K^+- and 0.86 ± 0.06 h^{-1} in Rb^+-media ($P < 0.001$). Addition of potassium channel blocker Ba^{2+} (1 mmol/l) to the medium caused a reduction in the rate of ^{86}Rb uptake by the lamprey red cells (Fig. 1*B*). The inhibitory effect of Ba^{2+} was two times greater in K^+-medium as compared to Rb^+-medium. In the presence of 1 mmol/l Ba^{2+}, the uptake of ^{86}Rb was linear over 60 min, and the rate coefficients were 0.72 ± 0.04 h^{-1} and 0.60 ± 0.03 h^{-1} in K^+- and Rb^+-media, respectively. The difference in Ba^{2+}-insensitive components of ^{86}Rb uptake between both media was relatively small but

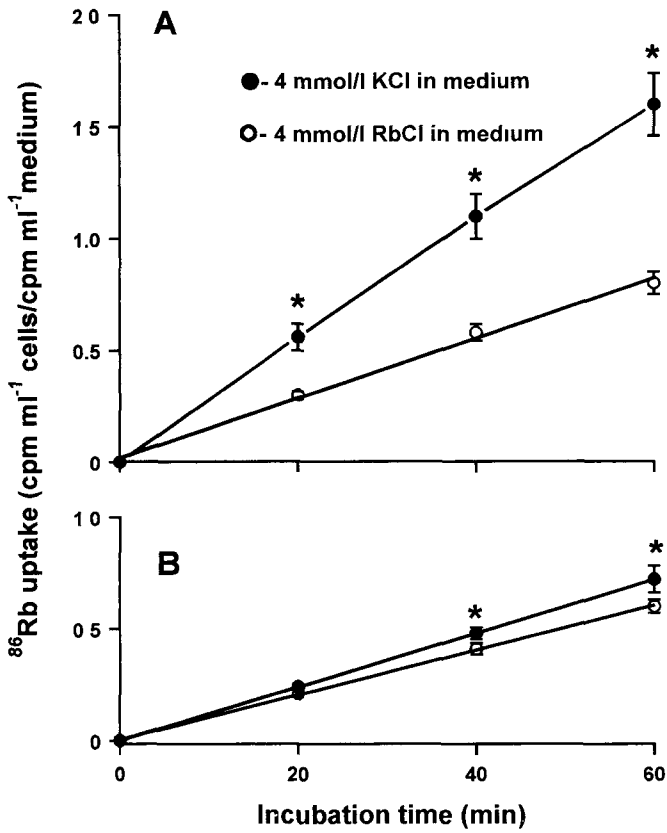

Figure 1. Time course of ^{86}Rb uptake by lamprey erythrocytes incubated in media containing 4 mmol/l K^+ or 4 mmol/l Rb^+ without (*A*) and with 1 mmol/l Ba^{2+} (*B*) Red cells were preincubated for 5 min in the respective media and ^{86}Rb was added at time zero ^{86}Rb uptake was measured as described in Materials and Methods ^{86}Rb uptake is expressed as the ratio of radioactivity (cpm) of 1 ml packed cells to radioactivity (cpm) of 1 ml medium The straight lines were fitted using linear regression analysis Data are means ± S E for 6 separate experiments * $P < 0.05$ as compared with Rb^+ medium (paired *t*-test)

statistically significant ($P < 0.01$, paired *t*-test) Thus, replacement of K^+ with Rb^+ in the incubation medium resulted in considerable decrease in ^{86}Rb uptake by the lamprey erythrocytes This decrement in ^{86}Rb uptake in Rb^+-medium was mainly accounted for by inhibition of the Ba^{2+}-sensitive component of ^{86}Rb transport across potassium channels in the lamprey erythrocyte membrane (Kirk 1991, Gusev et al 1992)

Total, Ba^{2+}- and ouabain-sensitive ^{86}Rb uptake

In the second set of experiments, ^{86}Rb uptake was measured in the lamprey erythrocytes incubated for 60 min in standard saline containing 4 mmol/l KCl or 4 mmol/l RbCl. Effects of both Ba^{2+} and ouabain on ion transport were examined in the experiments. The data summarized in Table 1 illustrate that the total and Ba^{2+}-insensitive ^{86}Rb uptake in the presence of K^+ were significantly greater than those in the presence of Rb^+. A marked decrease in the Ba^{2+}-sensitive ^{86}Rb influx was observed when K^+ in incubation medium was replaced with Rb^+ (Table 1). These data are in good agreement with the results of a previous series of experiments (Fig. 1). Addition of 0.1 mmol/l ouabain to the incubation medium in the presence of 1 mmol/l Ba^{2+} led to a significant reduction in ^{86}Rb uptake. The ouabain-sensitive influx of ^{86}Rb was only slightly higher in K^+-medium than in Rb^+-medium, the difference however being significant using paired statistical analysis. There was no statistical difference between the residual, (Ba^{2+} plus ouabain)-resistant, components of ^{86}Rb uptake by the lamprey red cells in both media (Table 1). These data clearly indicate that Ba^{2+}-sensitive potassium channels markedly discriminate between K^+ ions and Rb^+ ions.

Table 1. ^{86}Rb influx into lamprey erythrocytes incubated in K^+- and Rb^+-medium

Medium	Rate coefficient of ^{86}Rb influx (h^{-1})				
	Control	Ba^{2+}	Ouabain + Ba^{2+}	Ba^{2+}-sensitive	Ouabain-sensitive
KCl	1.48 ± 0.10	0.70 ± 0.05	0.082 ± 0.012	0.78 ± 0.10	0.62 ± 0.05
(n)	(13)	(13)	(6)	(13)	(6)
RbCl	0.82 ± 0.05**	0.59 ± 0.02**	0.068 ± 0.006	0.23 ± 0.04**	0.54 ± 0.05*
(n)	(13)	(13)	(6)	(13)	(6)

The erythrocytes were preincubated for 30 min in media containing (mmol/l): 140 NaCl; 1 $CaCl_2$; 10 Tris-HCl; 10 glucose and 4 KCl or RbCl (pH 7.4 at 20°C) without or with 1 mmol/l Ba^{2+} and Ba^{2+} plus ouabain. Then, ^{86}Rb was added and its uptake was measured over 60 min. The data represent mean ± S.E. for 6–13 independent experiments. * $P < 0.05$; ** $P < 0.01$ as compared with medium containing 4 mmol/l KCl (paired t-test).

^{41}K uptake by lamprey erythrocytes

In order to assess the true transport rate of K^+ ions across the lamprey erythrocyte membrane, the uptake of stable isotope ^{41}K by the red cells was studied. Lamprey erythrocytes were incubated in a medium containing 4 mmol/l ^{41}KCl, and ^{41}K accumulation in the red cells was determined using mass spectrometry (see Materials

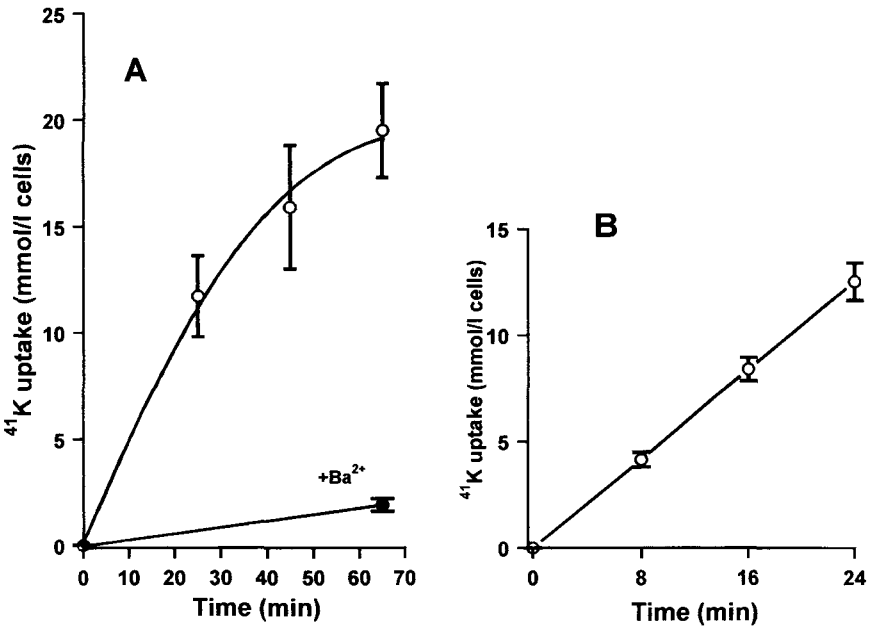

Figure 2. Time dependence of ^{41}K uptake by lamprey erythrocytes Red cells were preincubated for 5 min in a medium containing (mmol/l) 144 NaCl 1 $CaCl_2$ 10 Tris-HCl 10 glucose (pH 7 4 at 20 °C) Then at time zero 140 mmol/l ^{41}KCl solution was added to a final concentration of 4 mmol/l Samples of the suspension were taken at various time intervals over 65 min (*A*) and a 24 min period (*B*) Data points were fitted to single exponential function by a computer program (*A*) The straight line (*B*) has been drawn according to linear regression Values are mean ± S F of 3 (*A*) and 6 (*B*) separate experiments

and Methods) In the first series of experiments, the uptake of ^{41}K was measured in cells incubated for 25, 45, and 65 min As can be seen in Fig 2*A*, ^{41}K rapidly accumulated in the lamprey red cells within the first 25 min and the rate of ^{41}K uptake decreased during the following 40 min of cell incubation Thus, a non linear time dependency was observed during the 65 min period of cell incubation Therefore additional experiments were performed to assess the initial rate of ^{41}K uptake by measuring ^{41}K accumulation in cells incubated for 8, 16, and 24 min Fig 2*B* clearly illustrates that ^{41}K uptake was linear over the 24 min period The rate coefficient of ^{41}K uptake in lamprey erythrocytes was 8 1 ± 0 5 h^{-1} This value did not differ from the rate coefficient of ^{41}K uptake (7 0 ± 1 1 h^{-1}) calculated from the initial slope of the curve in the first series of experiments (Fig 2*A*) Blockade of potassium channels by 1 mmol/l Ba^{2+} was associated with a significant reduction in the rate of ^{41}K uptake by lamprey erythrocytes The uptake of ^{41}K in the presence of Ba^{2+} was linear over 120 min of cell incubation (1 7 ± 0 4 for 65 min

and 3 2 ± 0 5 mmol/l cells for 120 min) and the rate coefficient for K^+ uptake was 0 39 ± 0 11 h^{-1} (Fig 2 4) The mean intracellular concentration of K^+ measured by flame photometry was 56 9 ± 6 1 and 75 7 ± 4 5 mmol/l cells for the first and the second series of experiments respectively

Discussion

The results of the present study are consistent with the earlier report of Kuik (1991) and provide further evidence for a high selectivity of the potassium channels for K^+ over Rb^+ in the lamprey erythrocyte membrane Our comparative data on the rate of ^{86}Rb and ^{41}K influxes into the lamprey red cells via the Ba^{2+} sensitive potassium channels are summarized in Table 2 There is good quantitative agreement between the results obtained here with ^{41}K and those obtained previously with ^{43}K (Kuik 1991) At external K^+ concentration of 4 mmol/l the channel has a 9 fold preference for ^{41}K over ^{86}Rb and an 8 fold preference for ^{43}K over ^{86}Rb at external K^+ concentration of 5 mmol/l In addition we could show that ^{86}Rb influx into the lamprey red cells via potassium channels was significantly decreased after replacement of external K^+ with Rb^+ suggesting that Rb^+ interacts with the channel and behaves as a partial blocker It is evident that at the same K^+ and Rb^+ concentrations in the medium the tracer permeability of the potassium channels to Rb^+ must have only been about 3% of the permeability to K^+ The possibility cannot be ruled out that the effect of Rb^+ on the potassium channels may be due to changes in membrane potential In preceding studies (Kuik 1991 Gusev et al 1992) K^+ movement across the lamprey erythrocyte membrane was examined using ^{86}Rb as a tracer and in the presence of 1 4 mmol/l KCl in the media Under these conditions the Ba^{2+} inhibitable uptake of ^{86}Rb only reflects about 10% of the true permeability of the potassium channels to K^+ Recently an inwardly rectifying potassium current has been identified in lamprey erythrocytes using whole cell patch clamp technique (Virkki and Nikinmaa 1995) The authors

Table 2. Rate coefficients for Ba^{2+} sensitive influxes of ^{41}K and ^{86}Rb into lamprey erythrocytes (h^{-1})

	^{86}Rb influx		^{41}K influx
Medium	RbCl	KCl	^{41}KCl
	0 23 ± 0 04	0 78 ± 0 10*	7 28 ± 0 5**

The red cells were incubated in standard media containing 4 mmol/l RbCl KCl or ^{41}KCl The values are mean ± S E of 13 (^{86}Rb influx) and 6 (^{41}K influx) determinations * $P <$ 0 001 as compared with RbCl medium, ** $P <$ 0 001 as compared with ^{86}Rb influx

have found the K^+ channels to exhibit a much greater permeability to K^+ over Rb^+ as indicated by the reversal potential for channel currents.

The use of potassium radionuclides (^{42}K and ^{43}K) is limited due to their cost and a short half-life (about 11h and 23h, respectively). There have been numerous studies to demonstrate the possibility of using ^{86}Rb as an accurate quantitative marker of K^+ transport in a variety of cells and tissues (Beauge and Ortiz 1970; Cheval et al. 1991; Zhou and Wingo 1992). Therefore, ^{86}Rb is routinely employed as a substitute for K^+ to assay ion transport mechanisms in many types of cells for reasons of convenience (half-life of about 18 days) and cost. However, there is some evidence that Rb^+ may not be a perfect substitute for K^+ in all cases. Numerous patch-clamp and tracer studies have shown that some types of potassium channels do not discriminate between K^+ and Rb^+ ions, but other potassium channels have a considerably lower conductance to Rb^+ (for review see Hille 1973; Lattore and Miller 1983; Van Driessche and Zeiske 1985; Nichols and Lederer 1991). It has been known for a long time that the inward rectifier is slightly permeable to Rb^+ in a number of tissues such as frog skeletal muscle (Adrian 1964; Spalding et al. 1982), macrophages (Gallin and Livengood 1981), bovine artery endothelium (Silver et al. 1994) and guinea-pig ventricular cells (Matsuda 1996). The conductance of the inward rectifier channel for Rb^+ was extremely low, with a Rb^+/K^+ ratio of 0.03–0.04, while the relative Rb^+ permeability was only 0.3–0.5. These studies have suggested that Rb^+ interferes with the movement of K^+ through the channels and alters its kinetics. Similarly, potassium channels of some epithelial tissues have also been shown to exhibit a low conductance to Rb^+ but their permeability for Rb^+ is nearly the same as for K^+ (Gallacher et al. 1984; Gogelein et al. 1987; Bleich et al. 1990). Ca^{2+}-activated potassium channels of lymphocytes (Grissmer et al. 1993) and human erythrocytes (Christophersen 1991) display relatively small discrimination with respect to both the Rb^+/K^+ permeability (0.55–0.96) and Rb^+/K^+ conductance (0.36–0.60).

It is generally accepted that membrane transport carriers do not markedly discriminate between K^+ and Rb^+ ions. It is commonly admitted that Rb^+ can substitute for K^+ in Na-K-ATPase studies. In a variety of cells and tissues ^{42}K and ^{86}Rb yielded similar results when used as tracers to assay the activities of the Na-K-pump and cotransporters (Castronovo et al. 1968; Bernstein and Israel 1970; Schafer and Troutman 1986). However, the data of the present study indicate that the ouabain-sensitive ^{86}Rb influx in the lamprey red cells somewhat (by $\sim$ 16%) decreases after replacing 4 mmol/l KCl in the media with 4 mmol/l RbCl (Table 1). The results are consistent with the study of Warden et al. (1989) who have demonstrated a reduced activity of the Na,K-ATPase in several segments of rabbit nephron in the presence of 5 mmol/l Rb^+ ($\sim$ 20%) compared with 5 mmol/l K^+. Cheval and Doucet (1990) used the same approach to study the selectivity of ouabain-sensitive ^{86}Rb uptake in single segments of rat nephron by incubating

tubule preparations with 5 mmol/l Rb^+ or K^+. They have found that the active transport rate elicited by K^+ was 12–70% higher than that in the presence of Rb^+. In another study (Soltoff and Mandel 1984), the ouabain-sensitive rate of respiration in a suspension of renal proximal tubules was reduced to 83% in the presence of 5 mmol/l Rb^+ as compared with 5 mmol/l K^+. Thus the magnitude of the active transport will be underestimated when ^{86}Rb is used as a tracer and Rb^+ as a substitute for K^+ in external medium. When the incubation medium contains K^+ ions, ^{86}Rb can be considered to be an accurate marker for measuring the true activity of the Na,K-pump in the lamprey erythrocytes and other cell types. The K^+ and Rb^+ influxes in the lamprey red cells measured using ^{86}Rb as a tracer in the presence of Ba^{2+} plus ouabain were indistinguishable (Table 1), implying that the (Ba^{2+} + ouabain)-resistant pathway for the tracer movement does not discriminate between K^+ and Rb^+.

In summary, the present study clearly demonstrates that the potassium channels in the lamprey erythrocyte membrane have a strong preference for K^+ over Rb^+ as shown by the comparison of the Ba^{2+}-sensitive influxes between ^{41}K and ^{86}Rb. The ^{86}Rb influx via the channel decreased when all external K^+ was replaced with Rb^+, suggesting that Rb^+ may partially inhibit the channel conductance. In its selectivity properties the potassium channel in the lamprey erythrocyte membrane resembles a number of potassium channels so far studied in various cell types. Although there were quantitative differences between K^+ and Rb^+ transport in the lamprey erythrocytes, we found no evidence suggesting that these ions are transported by different mechanisms. It should be emphasized that when ^{86}Rb is used as a marker for study of the K^+ transport pathways, it is preferable to avoid substitution of Rb^+ for K^+ in incubation media. The present study shows the possibility of using stable ^{41}K isotope to investigate ion transport mechanisms across biological membranes.

Acknowledgements. The present study was made possible in part by grants No. NV0000 from the International Science Foundation, No. NV0300 from the International Science Foundation and the Russian Government.

References

Adrian R. H. (1964) The rubidium and potassium permeability of frog muscle membrane. J. Physiol. (London) **175,** 134–159.

Beauge L. A., Ortiz O. (1970) Rubidium, sodium and ouabain interactions on the influx of rubidium in rat red blood cells. J. Physiol. (London) **210,** 519–532.

Bernstein J. C., Israel Y. (1970) Active transport of Rb^{86} in human red cells and rat brain slices. J. Pharmacol. Exp. Ther. **174,** 323–329.

Bleich M., Schatter E., Greger R. (1990) The luminal K^+ channel of the thick ascending limb of Henle's loop. Pflugers Arch. **415,** 449–460.

Castronovo F P McIntyre P A Stern H S (1968) The standartization of a reproducible *in vitro* technique for the measurement of Rb uptake in the human erythrocyte Int J Appl Radiat Isotop **19,** 781—788

Cheval L , Barlet-Bas C Khadouri C Feraille E Marsy S , Doucet A (1991) K^+-ATPase-mediated Rb^+ transport in rat collecting tubule modulation during K^+ deprivation Amer J Physiol **260,** F800—F805

Cheval L , Doucet A (1990) Measurement of Na-K-ATPase-mediated rubidium influx in single segments of rat nephron Amer J Physiol **259,** F111 F121

Christophersen P (1991) Ca^{2+}-activated K^+ channel from human erythrocyte membranes single channel rectification and selectivity J Membrane Biol **119,** 75 –83

Fleishman D G Nikiforov V A Saulus A A (1992) Endogenous lithium determination in blood plasma and urine by isotope dilution mass spectrometry and preliminary isolation of lithium fraction using paper chromatography Biol Mass Spectrom **21,** 80—84

Gallacher D V Maruyama Y Petersen O H (1984) Patch-clamp study of rubidium and potassium conductances in single cation channels from mammalian exocrine acini Pflugers Arch **401,** 361—367

Gallin E K , Livengood D R (1981) Inward rectification in mouse macrophages evidence for a negative resistance region Amer J Physiol **241,** C9 C17

Gogelein H Greger R Schlatter E (1987) Potassium channels in the basolateral membrane of the rectal gland of Squalus acanthias Pflugers Arch **409,** 107—113

Grissmer S Nguyen A N Cahalan M D (1993) Calcium-activated potassium channels in resting and activated human T lymphocytes J Gen Physiol **103,** 601—630

Gusev G P Sherstobitov A O Skulskii I A (1992) Potassium transport in lamprey (*Lampetra fluviatilis*) erythrocytes evidence for K^+ channels Comp Biochem Physiol **101A,** 569 –572

Gusev G P Sherstobitov A O (1993) Evidence for furosemide-sensitive $Ca^{2+}(Mg^{2+})$-dependent Cl transport in red blood cells of lamprey (*Lampetra fluviatilis*) Comp Biochem Physiol **105A,** 769 775

Hille B (1973) Potassium channels in myelinated nerve Selective permeability to small cations J Gen Physiol **61,** 669—686

Ikehara T Yamaguchi H , Hosokawa K Takahashi A Miyamoto H (1993) Kinetic study on the effects of intracellular K^+ and Na^+ on the Na^+ K^+ Cl^- cotransport of HeLa cells by Rb^+ influx determination J Membrane Biol **132,** 115—124

Kirk K (1991) K^+ transport across the lamprey erythrocyte membrane characteristics of a Ba^{2+}- and amiloride-sensitive pathway J Exp Biol **159,** 303 324

Lattore R Miller C (1983) Conduction and selectivity in potassium channels J Membrane Biol **71,** 11—30

Matsuda H (1996) Rb^+ Cs^+ ions and the inwardly rectifying K^+ channels in guinea-pig ventricular cells Pflugers Arch **432,** 26—33

Nichols C G Lederer W J (1991) Adenosine triphosphate-sensitive potassium channels in the cardiovascular system Amer J Physiol **261,** H1675—H1686

Nikinmaa M Railo E (1987) Anion movements across lamprey (*Lampetra fluviatilis*) red cell membrane Biochim Biophys Acta **899,** 134—136

Schafer J A Troutman S L (1986) Effect of ADH on rubidium transport in isolated perfused rat cortical collecting tubule Amer J Physiol **250,** F1063— F1072

Silver M R Shapiro M S DeCoursey T E (1991) Effects of external Rb^+ on inward

rectifier K^+ channels of bovine pulmonary artery endothelial cells J Gen Physiol **103,** 519–548

Soltoff P, Mandel L J (1984) Active ion transport in the renal proximal tubule II Ionic dependence of the Na pump J Gen Physiol **84,** 623—642

Spalding B C, Swift J G, Senyk O, Horowicz P (1982) The dual effect of rubidium ions on potassium efflux in depolarized frog skeletal muscle J Membrane Biol **69,** 145–157

Van Driessche W, Zeiske W (1985) Ionic channels in epithelial cell membranes Physiol Rev **65,** 833—903

Virkki L V, Nikinmaa M (1995) Characterization of inwardly rectifying K conductance in lamprey (*Lampetra fluviatilis*) erythrocytes In Proc 4th Int Congress Comp Physiol Biochem Birmingham UK, Physiol Zool **68,** 185

Warden D H, Hayashi M, Schuster V L, Stokes J B (1989) K^+ and Rb^+ transport by the rabbit CCD Rb^+ reduces K^+ conductance and Na^+ transport Amer J Physiol **257,** F43–F52

Zhou X, Wingo C S (1992) Mechanisms of rubidium permeation by rabbit cortical collecting duct during potassium restriction Amer J Physiol **263,** F1134—F1141

Final version accepted September 16, 1997