An Amiloride-sensitive, Volume-dependent Na^+ Transport across the Lamprey (*Lampetra fluviatilis*) Erythrocyte Membrane

G. P. GUSEV and A. O. SHERSTOBITOV

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Thorez pr. 44, 194223 St. Petersburg, Russia

Abstract. Na^+ transport across the lamprey erythrocyte membrane was examined using ^{22}Na as a tracer. Both Na^+ influx and Na^+ efflux exhibit a wide variability among different lampreys due to amiloride-sensitive components. Addition of 1 mmol/l amiloride to incubation media resulted in a decrease of the Na^+ influx from 8.4 ± 0.9 to 5.5 ± 0.3 mmol/l cells/h ($n = 18$, $P < 0.001$), and of the rate coefficient of the Na^+ efflux from 0.50 ± 0.08 to 0.18 ± 0.02 h^{-1} ($n = 20$, $P < 0.001$). Cell shrinkage induced by addition of 100 mmol/l sucrose to an isotonic medium was associated with a significant increase in both the Na^+ influx and the Na^+ efflux which was entirely blocked by amiloride. The amiloride-sensitive components of the Na^+ fluxes were abolished by cell swelling in hypotonic media (210 mosm/kg water). In addition to activation of Na^+ influx by isoproterenol reported earlier (Gusev et al. 1992b), the present study demonstrated that isoproterenol also stimulated Na^+ efflux from the lamprey erythrocytes. Exposure of the red cells to an Na^+-free medium (replacing with $NMDG^+$) resulted in a significant enhancement in Na^+ efflux ($\sim$ 3-fold) which was completely inhibited by amiloride. Incubation of the red cells in an unbuffered saline was accompanied by a gradual raising of external pH_e measured with a pH-sensitive electrode, and the addition of 100 mmol/l sucrose to isotonic medium had no effect on pH_e. The amiloride-sensitive component of the Na^+ influx in the red cells incubated in the isotonic medium was unchanged when pH_e was lowered from 7.4 to 6.5 but it disappeared after raising pH_e to 8.0. The results of this study show that the lamprey erythrocyte membrane exhibits an amiloride-sensitive transport in either direction which is activated by cell shrinkage and isoproterenol, and is attenuated by cell swelling. No evidence was obtained for the contribution of well known Na^+-H^+ and Na^+-Na^+ exchangers to this amiloride-sensitive pathway under the conditions of our experiments.

Key words: Erythrocyte — Lamprey — Na^+ transport — Amiloride

Introduction

In a number of studies on the lamprey erythrocytes it has been shown that their membrane exhibits peculiar properties in the ion transport mechanisms. In contrast to erythrocytes of other species studied so far, the lamprey erythrocyte membrane does not contain the band 3 protein mediating an anion exchange (Ohnishi and Asai 1985; Nikinmaa and Railo 1987) but it contains potassium channels blocked by Ba^{2+}, quinine, tetraethylammonium and amiloride (Kirk 1991; Gusev et al. 1992a; Virkki and Nikinmaa 1995). It has also been found that amiloride inhibited Na^+ transport in the lamprey erythrocytes (Gusev et al. 1992b; Virkki and Nikinmaa 1994). This amiloride-sensitive Na^+ influx in lamprey red cells was found to be markedly stimulated by cell shrinking. Moreover, the amiloride-sensitive component of Na^+ influx in lamprey erythrocytes was activated by isoproterenol or cAMP. Treatment of the red cells with dinitrophenol and incubation in the presence of propionate was also associated with an increase in Na^+ influx. On the basis of these results, it was proposed that the lamprey erythrocyte membrane contains an amiloride-sensitive Na^+/H^+ exchanger which is activated by cell shrinkage and catecholamines. A similar Na^+/H^+ antiporter has been documented in many cell types including erythrocytes (Jennings et al. 1986; Frelin et al. 1988; Semplicini et al. 1989; Motais et al. 1992).

The present work was focused on investigation of Na^+ efflux from the lamprey red cells as well as on the effects of cell volume and extracellular pH on Na^+ influx. Unexpectedly, we found the presence of an amiloride-sensitive component of Na^+ efflux which was stimulated by cell shrinking, by isoproterenol and removal of external Na^+. We also showed that cell swelling attenuated the amiloride-sensitive components of the Na^+ influx and the Na^+ efflux. Moreover, cell shrinkage was not associated with H^+ efflux from the lamprey red cells incubated in an unbuffered medium. The present study clearly demonstrates the existence of bidirectional amiloride-sensitive Na^+ transport via the lamprey erythrocyte membrane.

Materials and Methods

Animals

All experiments were performed on river lampreys (*Lampetra fluviatilis*) weighing 40–70 g. The animals were kept in aquaria with aerated tap water at 2–4 °C for several months (from November to March). The bath water was periodically replaced by fresh tap water.

Preparation of cells

After rapid decapitation blood was drawn into a tube containing ice-cold heparinized saline The cells were sedimented by centrifugation (2700 × *g* for 5 min

at 4°C), the supernatant and the buffy coat were removed by aspiration. Then the red cells were washed twice in ice-cold saline and suspended at 30–40% hematocrit in saline. The cell suspension was kept at room temperature for 15 min before measuring Na^+ transport.

Measurement of Na^+ influx

Na^+ influx was determined from the uptake of ^{22}Na. To study the effects of cell shrinkage, the incubaton media contained 140 mmol/l NaCl, and hypertonic media additionally contained 90 mmol/l sucrose. To examine the effects of cell swelling, hypotonic media contained 96 mmol/l NaCl and 90 mmol/l sucrose were added to isotonic media. Na^+ -free medium was prepared by replacing Na^+ with isomolar N-methyl-D-glucamine ($NMDG^+$). All media also contained (mmol/l): 4 KCl; 1 $CaCl_2$; 1 $MgCl_2$; 10 Tris-HCl; 10 glucose (pH 7.4 at 20°C). Suspension of washed cells was added to the incubation media to a final hematocrit of 2–3%. After 5 min preincubation, ^{22}Na was added to the cell suspension ($\sim$ 2 μCi/ml medium). At definite intervals, 1 ml samples of the suspension were removed and injected into 10 ml ice-cold saline. After centrifugation (2700 $\times$ g for 1 min at 4°C), a supernatant aliquote was taken to determine the radioactivity of the medium.The red cells were washed twice with ice-cold saline and were lysed in distilled water to measure radioactivity. The Na^+ uptake was expressed as mmol per liter of original volume of packed cells.

Measurement of Na^+ and H^+ efflux

The red cells were incubated for 60 min at room temperature in isotonic saline containing about 20 μCi/ml ^{22}Na. The cells were then sedimented, washed five times with saline and resuspended in the corresponding isotonic medium at a hematocrit of 40%. Aliquots of the suspension were added to flux media to a final hematocrit of 1–2%, then 1 ml samples were removed at 3 min (hypertonic media) or 10 min intervals (iso- and hypotonic media) and immediately centrifuged for 1 min. Supernatant samples of 0.8 ml were taken to measure medium radioactivity. Efflux rate coefficients were calculated from the slope of the plot of $-\ln(1 - A_t/A_\infty)$ versus time, where A_t represents the activity in the medium at time (t), and A_∞ is the activity at isotopic equilibrium.

To determine H^+ efflux, the lamprey erythrocytes were washed with unbuffered saline containing (mmol/l): 140 NaCl; 4 KCl; 1 $CaCl_2$; 1 $MgCl_2$; 10 glucose. Then, the cells were incubated in the same saline at a hematocrit of 5%, and extracellular pH was recorded using a glass pH-sensitive electrode. After a control period of 30 min sucrose was added to the cell suspension to a final concentration of 100 mmol/l.

Reagents

Amiloride, ouabain and N-methyl-D-glucamine were obtained from Sigma Chemical

Co. (St. Louis, Mo, USA). ^{22}Na was purchased from ISOTOP (Russia). All other chemicals were of analytical reagent grade.

Statistics

All results are given as mean values ± S.E.M. Statistical significance was determined by the Student's *t*-test for paired observations. Rate coefficients of ^{22}Na efflux were calculated from linear regression fits using a computer program.

Results

Effects of cell shrinkage on Na^+ influx and efflux

In a series of experiments, we studied the time course of ^{22}Na uptake by the lamprey red cells incubated in isotonic and hypertonic media without and with 1 mmol/l amiloride. As can be seen from Fig. 1, the addition of 100 mmol/l sucrose to standard saline markedly stimulated Na^+ uptake by lamprey erythrocytes. Na^+ uptake was linear over the first 20–30 min of cell incubation. The average uptake values for the first 20 min were 4.3 ± 1.0 and 14.1 ± 1.7 mmol/ l cells ($P < 0.001$) in isotonic and hypertonic media, respectively. Addition of 1 mmol/l amiloride to incubation media led to a significant decrease in Na^+ uptake and a complete inhibition of the shrinkage-induced Na^+ influx. These results are in agreement with our previous study (Gusev et al. 1992b).

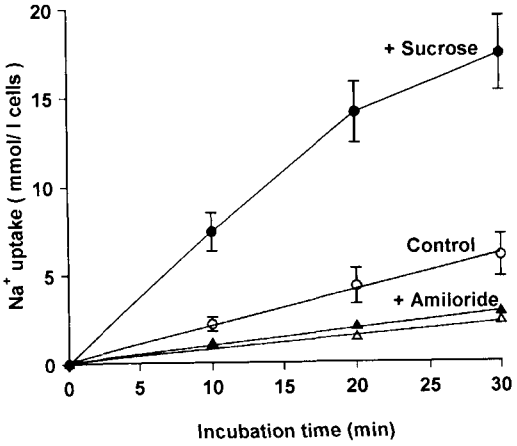

Figure 1. Effect of cell shrinkage on time course of Na^+ uptake by red cells. The red cells were incubated in isotonic (empty circles) and hypertonic (filled circles) media without or with 1 mmol/l amiloride (empty and filled triangles). Values are means ± S.E.M. for 4 paired experiments. Error bars were omitted when smaller than symbols.

In a next series of experiments, Na^+ efflux from ^{22}Na preloaded red cells was examined in isotonic and hypertonic media in the presence or in the absence of 1 mmol/l amiloride. As can be seen in Fig. 2, cell shrinkage resulted in a sharp acceleration of ^{22}Na efflux from lamprey erythrocytes which was totally abolished in the presence of amiloride. The rate coefficient for ^{22}Na efflux was also reduced

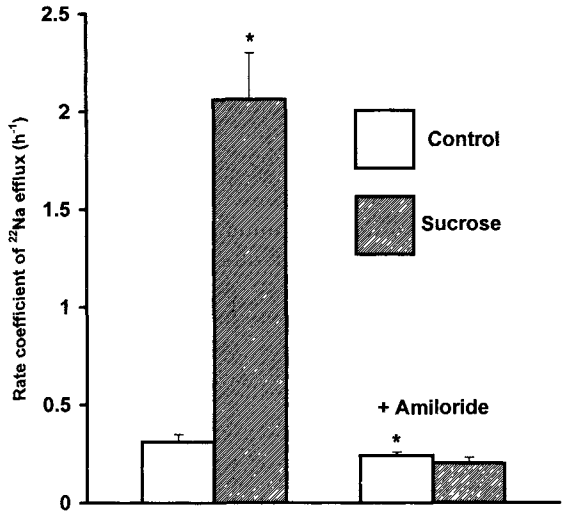

Figure 2. Effect of cell shrinkage on ^{22}Na efflux from red cells. The red cells preloaded with ^{22}Na were incubated in corresponding media, and rate coefficients of ^{22}Na efflux were measured as described in Materials and Methods. Results are expressed as means ± S.E.M. for 9 paired experiments. * $P < 0.01$ as compared with control in the absence of amiloride.

when amiloride was added to red cells incubated in control isotonic medium. Thus, these experiments indicated the existence of an amiloride-sensitive Na^+ transport through the lamprey erythrocyte membrane which was substantially activated by cell shrinkage.

Effects of cell swelling on Na^+ fluxes

To characterize further the amiloride-sensitive Na^+ transport in lamprey erythrocytes, experiments were done in which cell swelling was induced. In paired experiments the lamprey erythrocytes were incubated in isotonic and hypotonic media of the same composition except 90 mmol/l sucrose in isotonic medium. Na^+ influx into the red cells was determined from ^{22}Na uptake for 30 min which remained linear throughout. Fig. 3*A* shows the results of these experiments. Swelling the lamprey erythrocytes in a hypotonic medium led to a significant (by 44%) reduction in Na^+ influx. Addition of 1 mmol/l amiloride to isotonic medium also caused a decrease in Na^+ influx but amiloride had no effect in hypotonic medium. There was no difference between Na^+ influxes in red cells incubated in both media in the presence of amiloride. It was evident that cell swelling was associated with a complete inhibition of the amiloride-sensitive Na^+ influx in lamprey erythrocytes.

^{22}Na efflux was studied in similar paired experiments on red cells incubated in isotonic and hypotonic media. As can be seen in Fig. 3*B*, the rate coefficient of Na^+ efflux was significantly diminished (by 26%) in swollen cells as compared to control cells. Na^+ efflux from lamprey erythrocytes was inhibited by amiloride in isotonic, but not in hypotonic medium. As in the case of Na^+ influx, swelling of lamprey erythrocytes was associated with the disappearance of the amiloride-sensitive component of the Na^+ efflux. Results of these experiments suggest the

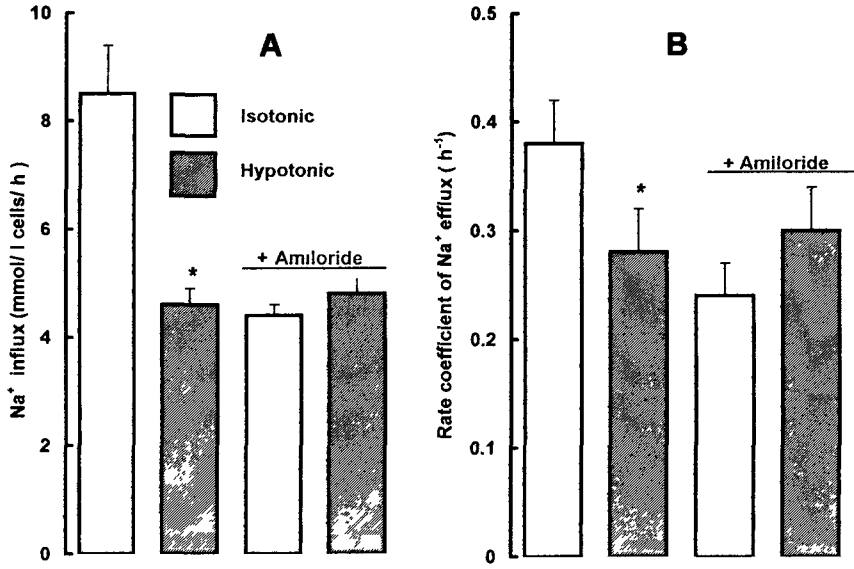

Figure 3. Effects of cell swelling on Na^+ influx (*A*) and Na^+ efflux (*B*). The results represent means ± S.E.M. of two series of paired experiments: *A*. Na^+ influx ($n = 5$) and *B*. Na^+ efflux ($n = 6$). Na^+ influx was determined from ^{22}Na uptake for 30 min. Rate coefficients were calculated as described in Materials and Methods. * $P < 0.01$ as compared to isotonic media.

presence of an amiloride-sensitive pathway for Na^+ transport across the lamprey erythrocyte membrane which is activated by cell shrinkage, and is inhibited by cell swelling.

Na^+ efflux from red cells in Na^+-free medium

In order to assess the presence of the Na^+-Na^+ exchange mechanism in the lamprey erythrocytes, we studied Na^+ efflux from the cells in a nominally Na^+-free medium. Replacing external Na^+ by N-methyl-D-glucamine ($NMDG^+$) was accompanied by a 3.4-fold increase in Na^+ efflux in isotonic medium (Fig. 4). An additional activation of Na^+ efflux from lamprey erythrocytes was observed after addition of 100 mmol/l sucrose to the Na^+-free medium. The stimulation of Na^+ efflux in isotonic and hypertonic Na^+-free media was entirely inhibited by amiloride. In the presence of amiloride the rate coefficient of Na^+ efflux in Na^+-free medium was close to that in standard saline (Fig. 3*B*). Thus, removal of external Na^+ caused a significant elevation in Na^+ efflux mediated via an amiloride-sensitive pathway. The stimulatory effect of cell shrinkage on Na^+ efflux was independent of the presence of external Na^+.

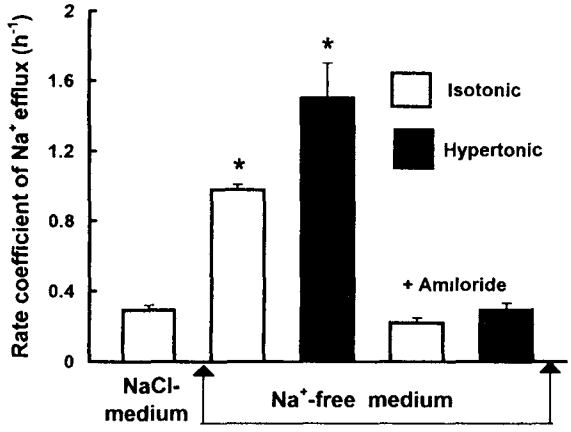

Figure 4. Effect of removal of external Na^+ on ^{22}Na efflux. The red cells preloaded with ^{22}Na were washed with standard saline or Na^+-free saline (equimolar replacing with $NMDG^+$) and incubated in the media without or with 100 mmol/l sucrose (hypertonic medium) and 1 mmol/l amiloride. Values are means ± S.E.M. for 5 paired experiments. * $P < 0.001$ vs. NaCl-medium.

Effect of isoproterenol on Na^+ efflux

In a previous study (Gusev et al. 1992b) we could show that 10^{-5} mol/l beta-adrenergic agonist isoproterenol caused an increase in Na^+ influx which was blocked by amiloride. Therefore it was of interest to test the possible effect of isoproterenol on Na^+ efflux from the lamprey red cells. Erythrocytes preloaded with ^{22}Na were incubated in standard medium without and with 10 μmol/l isoproterenol, and the rate of ^{22}Na efflux was measured. In 6 separate experiments the rate coefficient of Na^+ efflux was 0.28 ± 0.03 h^{-1} for control cells and 0.57 ± 0.05 h^{-1} ($P < 0.001$) in the presence of isoproterenol. It was evident that isoproterenol activated Na^+ transport across the lamprey erythrocyte membrane in both directions.

Effect of Ba^{2+} and ouabain on Na^+ transport

It has been well documented that treatment of the lamprey erythrocytes with 1 mmol/l Ba^{2+} leads to inhibition of K^+ channels and membrane depolarization (Kirk 1991; Gusev et al. 1992a). Therefore, Ba^{2+} could have an indirect influence on Na^+ efflux from the red cells due to cell depolarization. In five paired experiments, no changes in the ^{22}Na efflux from lamprey erythrocytes were found in the presence of 1 mmol/l Ba^{2+} (0.45 ± 0.13 vs. 0.43 ± 0.12 h^{-1} for control cells). Exposure of the red cells to 0.1 mmol/l ouabain resulted in a reduction of ^{22}Na extrusion down to 0.33 ± 0.03 h^{-1} as compared with 0.44 ± 0.02 h^{-1} ($P < 0.01$, $n = 4$). Ba^{2+} had also no effect on the Na^+ influx in the red cells (10.4 ± 0.4 vs. 11.9 ± 0.5 mmol/l/h in control, $n = 5$).

Effects of cell shrinkage on H^+ efflux and intracellular ion concentration

Many studies on erythrocytes and other cell types have demonstrated that cell shrinkage and catecholamines can activate Na^+/H^+ exchange which is associated

with net H^+ loss from the cells and with intracellular Na^+ accumulation. We carried out experiments to ascertain the involvement of Na^+-H^+ exchange in the observed effects of cell shrinkage. Lamprey erythrocytes were incubated in an unbuffered nominally HCO_3^- free saline, and pH of extracellular medium (pH_e) was recorded with a glass pH-sensitive electrode. The results of these experiments are presented in Fig. 5. Addition of red cells washed with unbuffered saline to the incubation medium was associated with a small shift in pH_e in the alkaline direction followed by a further gradual increase in pH_e throughout the incubation. It was quite evident that cell shrinkage had no effect on changes of pH_e. The shift in pH_e during 30 min of cell incubation was 0.28 ± 0.05 units for the control period in isotonic medium and 0.17 ± 0.04 pH units after addition of 100 mmol/l sucrose.

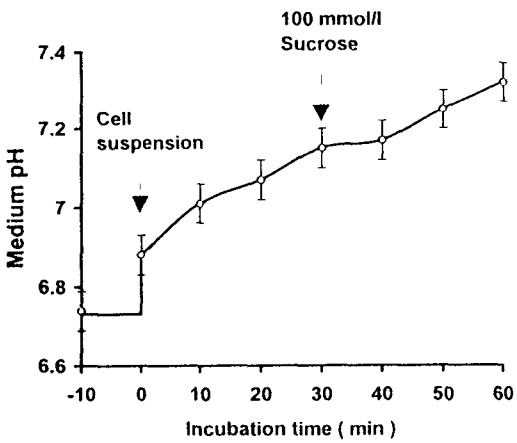

Figure 5. Changes in external pH_e of cell suspension in unbuffered saline. The red cells were washed and incubated in a buffer-free saline. External pH_e was measured with a pH-sensitive electrode. Each point represents mean ± S.E.M. of 7 experiments.

The lack of Na^+/H^+ exchange in the lamprey erythrocytes during hypertonic cell shrinkage was also supported by the results of measurement of cell ion composition using flame photometry. Intracellular Na^+ and K^+ concentrations were determined in the lamprey red cells incubated for 60 min in standard isotonic saline and a hypertonic medium containing 100 mmol/l sucrose (Table 1). There were no differences in the intracellular Na^+ and K^+ contents between control and shrunken cells when the ion content was expressed per liter original volume of packed cells. Similar results were obtained in another series of experiments when the ion concentrations were calculated per kg dry cell weight (data not shown). As seen from Table 1, treatment of the red cells with 1 mmol/l amiloride did not affect the cell ion concentrations in the erythrocytes incubated in both media.

Effect of pH_e changes on Na^+ influx

In mammalian erythrocytes, as in some other cell types, the Na^+-H^+ exchanger is dormant until a transmembrane H^+ gradient is created. Therefore, we evaluated

Table 1. Effect of cell shrinkage and amiloride on ion concentration (mmol/l original volume of packed cells)

Ion	Isotonic	Hypertonic	+ 1 mmol/l amiloride	
			Isotonic	Hypertonic
Na^+	21.1 ± 4.8	19.1 ± 3.2	21.0 ± 4.9	19.8 ± 3.8
K^+	74.5 ± 5.6	72.5 ± 3.8	75.6 ± 3.8	70.5 ± 4.4

The lamprey erythrocytes were incubated for 60 min in isotonic and hypertonic (+ 100 mmol/l sucrose) media without or with 1 mmol/l amiloride. After incubation the cells were washed with cold solutions containing 110 mmol/l or 140 mmol/l $MgCl_2$ buffered with 10 mmol/l Tris-HCl. Data present means ± S.E.M. of 3 independent experiments.

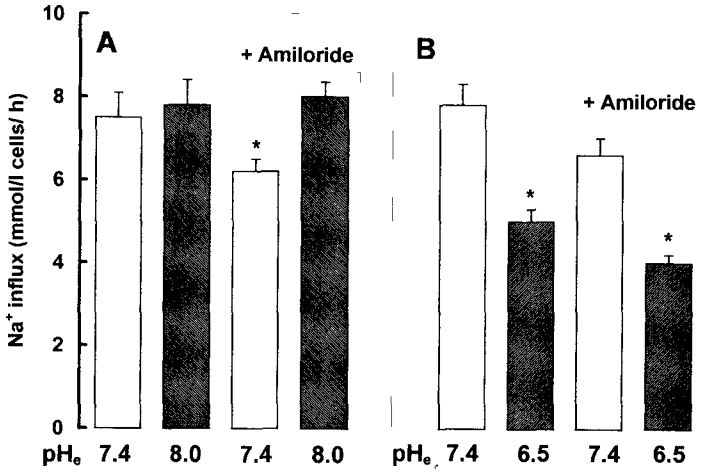

Figure 6. Dependency of Na^+ influx on external pH_e. The red cells were washed and incubated in isotonic saline buffered with 10 mmol/l Tris-HCl at pH 7.4 and pH 8.0 (*A*) or buffered with 5 mmol/l Na phosphate salts at pH 7.4 and pH 6.5 (*B*). Values are means ± S.E.M. for 7 paired experiments. * $P < 0.01$ as compared with no amiloride.

the effects of changes in external pH_e on the amiloride-sensitive Na^+ influx in the lamprey red cells. Na^+ influx measurements were made for red cells incubated in standard saline buffered with Tris-HCl at pH_e 7.4 and 8.0 or in saline buffered with phosphate salts at pH_e 7.4 and 6.5. Fig. 6 illustrates the results of these paired experiments. There was no difference between the Na^+ influxes in the red cells exposed to the salines at pH_e 7.4 in the presence of either buffer. Upon acidifying the medium to pH_e 6.5 a significant decline was measured in total and amiloride-insensitive Na^+ influx ($P < 0.001$) while the amiloride-sensitive component was

unchanged (1.2 ± 0.2 and 1.5 ± 0.3 mmol/l/h at pH_e 7.4 and 6.5, respectively). On the other hand, the medium alkalinization had no effect on total Na^+ influx but it entirely blocked the amiloride-sensitive Na^+ influx. The amiloride-sensitive Na^+ influx in the lamprey erythrocytes exposed to standard Tris-buffered medium (1.3 ± 0.3 mmol/l/h) did not differ from that in the red cells incubated in the saline buffered with phosphate buffer at pH_e 7.4 and 6.5.

Discussion

We could show in our previous work (Gusev et al. 1992b) the presence of an amiloride-sensitive Na^+ influx into the lamprey erythrocytes which was stimulated by cell shrinkage and isoproterenol. Similar to our findings, Virkki and Nikinmaa (1994) have also demonstrated the activation of amiloride-sensitive Na^+ influx in the lamprey erythrocytes by cell shrinking and cAMP. It was concluded on the basis of these results that the lamprey erythrocyte membrane contains Na^+-H^+ exchanger. In the present study we could confirm the stimulatory influence of cell shrinkage on the amiloride-sensitive Na^+ influx but also demonstrated an opposite effect of cell swelling on this transport pathway in the lamprey red cells. Data of the present work provide the first evidence for Na^+ efflux from the lamprey erythrocytes also being mediated by an amiloride-sensitive mechanism. The amiloride-sensitive component of the Na^+ efflux from the red cells is enhanced by cell shrinking and by removal of external Na^+, but is totally inhibited by cell swelling. Thus, the results of the present study suggest the existence of an amiloride-inhibitable pathway of Na^+ transport across the lamprey erythrocyte membrane in either direction. This amiloride-sensitive Na^+ transport is raised after exposure of the red cells to hypertonic medium and inhibited during cell swelling. Moreover, the amiloride-sensitive Na^+ movement is modulated by catecholamines, since a treatment of the red cells with isoproterenol accelerates both the Na^+ influx and efflux.

Under the conditions of our assays the amiloride-sensitive components of the Na^+ influx and efflux were observed in a majority of our experiments. However, there was a wide variability in the magnitudes of these components among the lampreys studied. In one series of paired experiments (Fig. 1) the amiloride-sensitive Na^+ influx was 4.7 ± 0.4 mmol/l/h whereas it was 1.3 ± 0.3 mmol/l/h in the studies of pH effects on Na influx (Fig. 6). In a third series of experiments the amiloride-sensitive component of the Na^+ influx was 3.1 ± 0.2 mmol/l/h ($n = 7$). Taken as a whole, the average value for the amiloride-sensitive Na influx in the lamprey erythrocytes was found to be 3.0 ± 0.2 mmol/l/h ($n = 18$). The rate coefficients for Na efflux measured in 20 lampreys varied from 0.20 to 1.6 h^{-1} for the control cells and from 0.07 to 0.30 h^{-1} for the amiloride-treated cells. Marked differences in the magnitude of Na transport from the lamprey red cells are accounted for by mainly an amiloride-sensitive component which was found to be within 0–1.3 h^{-1},

and the average value of the amiloride-sensitive efflux of Na^+ was 0.32 ± 0.06 h^{-1} ($n = 20$). Only in 3 out of 20 experiments amiloride had no effect on Na^+ extrusion from the lamprey erythrocytes.

Numerous studies have demonstrated that amiloride and its analogs are common blockers of several Na^+ transporters (for a review see Grinstein and Rothstein 1986; Sariban-Sohraby and Benos 1986; Frelin et al. 1988). The amiloride-sensitive Na^+/H^+ exchanger and Na^+ channels are characteristic transport systems of many animal tissues. Na^+/H^+ exchange is nearly ubiquitous among vertebrate cells, including human red cells and nucleated erythrocytes of animals (Jennings et al. 1985; 1986; Escobales and Canessa 1986; Semplicini et al. 1989; Motais et al. 1992). Under physiological conditions the Na^+/H^+ in untreated cells is usually quiescent. Although our previous study (Gusev et al. 1992b) and the work of Virkki and Nikinmaa (1994) have suggested the presence of Na^+/H^+ exchange in the lamprey erythrocyte membrane, the results of the present study do not support this idea. This conclusion is based on the following evidence: first, cell shrinking and isoproterenol activated both the amiloride-sensitive Na^+ influx and the amiloride-sensitive Na^+ efflux; second, cell shrinking was not associated with H^+ loss from the cells in an unbuffered saline; third, Na^+ cell content (mmol per kg dry weight) remained constant after cell shrinkage; and finally, the amiloride-sensitive Na^+ influx remained unaffected by external acidification and was inactivated after alkalinization of the incubation medium. The results of our investigation are in a good agreement with the report of Virkki and Nikinmaa (1994), in which osmotic shrinkage of lamprey red cells was not accompanied by any change in the intracellular pH and in the cell ion content. Similar to our findings, these authors did not find any effect of medium alkalinization on total Na^+ influx in the lamprey erythrocytes. The experiments with addition of 50 mmol/l propionate to the incubation medium in the later study (Fig. 2, Virkki and Nikinmaa 1994) did not provide convincing evidence for H^+ efflux from the red cells via the Na^+/H^+ exchanger. Intracellular acidification from pH $\sim$ 8.0 to pH $\sim$ 7.3 after cell incubation in a propionate containing medium remained unchanged for the first 30 min when maximum accumulation of Na^+ in the cells was observed. The enhancement of cell Na^+ content under these conditions may be alternatively attributed to the effect of propionate itself on Na^+ transport. This Na^+ transport coupled with anions of weak acids is well documented in plasma membrane of many cell types (see e.g., Bergman et al. 1989). At present it cannot be excluded that Na^+/H^+ exchange system is present in the lamprey erythrocyte membrane, but further studies are needed to elucidate this possibility.

The amiloride-sensitive Na^+ transport across the lamprey erythrocyte membrane found in our studies may be mediated by either a carrier system or by a channel mechanism. The well-known Na^+/Na^+ exchanger is a possible candidate for such a carrier-mediated Na^+ transport. However, we failed to obtain evidence for

the participation of the Na^+/Na^+ exchange mechanism in Na^+ movement through the lamprey erythrocyte membrane. Efflux of ^{22}Na from the lamprey erythrocytes was accelerated after replacing Na^+ with $NMDG^+$ in the incubation medium (Fig. 4). In contrast, in mammalian red cells the Na^+ efflux has been shown to be activated by extracellular Na^+ owing to the functioning of the Na^+/Na^+ exchanger (Dunn and Grant 1974; Jennings et al. 1985; Escobales and Figueroa 1991). Numerous studies on epithelial tissues have demonstrated the presence of amiloride-sensitive Na^+ channels which are voltage dependent (for a review see Sariban-Sohraby and Benos 1986; Garty and Edelman 1988). Similar Na^+ channels have also been found in some nonepithelial tissues (Bubien et al., 1994). The amiloride-sensitive transport of Na^+ via the lamprey erythrocyte membrane appears not to be a conductive pathway. The suggestion is based on the inability to induce changes in Na^+ influx and efflux in the presence of Ba^{2+}, a blocker of K^+ channels, which causes membrane depolarization (Kirk 1991). It should be noted that amiloride itself inhibits K^+ channels in the lamprey erythrocytes (Kirk 1991; Gusev et al. 1992a) thereby causing membrane depolarization.

There was some evidence for an undetermined amiloride-inhibitable Na^+ transport in either direction through the plasma membrane of erythrocytes and some other tissues. In human red blood cells, an outward H^+ gradient activated Na^+ influx via Na^+/H^+ exchange, but also stimulated Na^+ efflux, which was partially inhibited by amiloride (Escobales and Canessa 1986; Semplicini et al. 1989). Escobales and Canessa (1985) have also demonstrated an amiloride-sensitive Na transport across the human erythrocyte membrane which was activated by increasing intracellular Ca^{2+} concentration. Similar to lamprey erythrocytes in our experiments, ^{22}Na efflux from rabbit (Jennings et al. 1986) and pig erythrocytes (Sergeant et al. 1989) was stimulated four to five-fold by cell shrinkage, and this stimulation was nearly completely inhibited by amiloride. The amiloride-sensitive system in shrunken rabbit and pig erythrocytes can mediate net Na movement in either direction. Moreover, an inward H^+ gradient did not activate the ^{22}Na efflux from rabbit red cells. In contrast to lamprey erythrocytes, an activation of net H^+ loss from the rabbit and pig red cells was observed during osmotic shrinking. A certain amiloride-inhibitable pathway of Na^+ influx has been described in ground squirrel and guinea pig red blood cells (Zhou and Willis 1989). In studies on thymic lymphocytes (Grinstein et al. 1984) it was shown that about 20–30% of the total Na^+ influx and efflux was inhibited by amiloride. A recent work by Wehler et al. (1995) showed that hypertonic stress of rat hepatocytes led to a considerable increase in cell membrane Na^+ conductance which was completely blocked by amiloride. In the investigations cited above the amiloride- sensitive transport of Na^+ across the plasma membranes of erythrocytes and other tissues could be attributed to an Na^+/H^+ exchange mechanism operating in an unusual mode. Indeed, recent studies have shown the ability of the Na^+/H^+ antiporter to exhibit

both electroneutral and conductive Na^+ transport in the basolateral membrane of turtle colon epithelium (Post and Dawson 1992, 1994). Similar to our findings on lamprey erythrocytes, this amiloride-sensitive Na^+ transport associated with Na^+ conductance, Na^+/Na^+ and Na^+/H^+ exchange in the turtle colon epithelium was markedly attenuated after cell swelling and activated by cell shrinkage. The relative proportion of exchange (Na^+/H^+ and Na^+/Na^+) and conductive pathways is believed to be determined by the magnitude and orientation of the ion gradients and membrane potential. In our experiments, the acceleration of ^{22}Na efflux from lamprey erythrocytes bathed in an Na^+-free medium (Fig. 4) appears to be associated with a net Na^+ loss from the cells. It is of interest to note that the Na^+/H^+ antipoter can also mediate transmembrane H^+ currents (Demaurex et al. 1995).

In conclusion, the data obtained in the present study clearly indicate the existence of a bidirectional amiloride-sensitive Na^+ transport through the lamprey erythrocyte membrane. This amiloride-sensitive movement of Na^+ in either direction is activated by cell shrinking and entirely inhibited by cell swelling. Similar to many transporters, it is also modulated by catecholamines. The amiloride-sensitive Na^+ transport may represent either an altered operating mode of the Na^+/H^+ exchanger or an undefined transport carrier. Under the conditions of our experiments, we cannot detect any activity of the Na^+/H^+ exchanger in the lamprey erythrocytes. The existence of the Na^+/H^+ exchanger in these cells remains to be defined in further studies.

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