Biochimica et Biophysica Acta 856 (1986) 388–391
Elsevier

BBA 70294

BBA Report

An unusual pattern of Na^+ and K^+ movements across the horse erythrocyte membrane

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(Received September 24th, 1985)
(Revised manuscript received January 13th, 1986)

Key words: Erythrocyte; Na^+/Li^+ countertransport; Na^+ pump, Na^+-K^+ cotransport; (Human, horse)

Marked differences in the activities of three monovalent cation transport systems in horse versus human erythrocytes are reported. Whereas horse erythrocytes exhibit a 6-fold higher sodium-lithium countertransport, the unidirectional flux of potassium through the sodium pump is 3–4-times slower and the sodium-potassium cotransport system cannot be detected. In spite of this, horse and human cells are able to maintain similar Na^+ and K^+ gradients.

It is now well documented that in addition to the ouabain-sensitive Na^+ pump, at least two other systems are involved in monovalent cation movement in human erythrocytes. One the these systems is the Cl^- dependent Na^+-K^+ cotransport, which is inhibited by loop diuretics such as furosemide and bumetanide [1,2,3], the other is the Na^+-Na^+ exchange system, which can also catalize Na^+-Li^+ or Li^+-Li^+ exchange and is inhibited by phloretin [4,5].

In spite of several reports dealing with the transport mechanism responsible for these ouabain-resistant Na and K fluxes [1,3,6–9], their physiological role still remains poorly understood. Recently, it has been proposed that the Na^+/K^+ cotransport system may be involved in K^+ homeostasis and volume regulation, but the precise mechanism has not yet been established [10,11].

Here we present experimental evidence showing that the relative activities of the Na and K transport systems in horse erythrocytes differ significantly from those found in human red blood cells; the horse erythrocyte is nevertheless capable of maintaining similar Na^+ and K^+ gradients as the human red cell. We believe therefore, that horse erythrocytes may serve as a powerful complementary tool for investigating the relationship between these activities and the Na, K and water balance.

Blood samples from nine healthy trained adult Thoroughbred race horses, age 3–4 years were used in the study. The samples were collected in heparinized syringes from the yugular vein and kept in ice until centrifugation; usually the blood was centrifuged between 30–40 min after drawn. After removing the plasma and buffy coat, the red cells were washed three times with an isoosmotic solution containing 75 mM $MgCl_2$/60 mM sucrose/11 mM glucose/ 15 mM Tris-HCl (pH 7.4) at 4°C (magnesium buffer).

The measurement of Na^+-Li^+ exchange was performed essentially as described by Canessa et al. [12] with minor modifications. Briefly, the washed red cells were loaded with Li by incubating for 3 h at 37°C in a solution containing LiCl (140 mM), Tris-HCl (15 mM, pH 7.4) and glucose (11 mM) at 20% hematocrit. The cells were then

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washed four times with magnesium buffer to eliminate external Li. Lithium efflux was measured by incubating cells in either magnesium buffer (sodium free medium) or a solution containing NaCl (140 mM), Tris-HCl (15 mM, pH 7.4), and glucose (11 mM) (sodium-enriched medium) at 10% hematocrit and 37°C. In both cases ouabain was present at 0.1 mM. Aliquots of the cell suspension were taken at time intervals and added to tubes containing 0.3 ml dibutylphthalate, an organic layer which after 1 min centrifugation at $13000 \times g$ separates the loaded cells from the surrounding medium. Li was determined in the supernatant above the organic layer by means of atomic absorption spectrophotometry (Perkin-Elmer Model 303).

Unidirectional K^+ influx was estimated by incubating washed cells at 10% hematocrit and 37°C in a solution containing: NaCl (146 mM), KCl (4 mM), Tris-HCl (8 mM, pH 7.4), glucose (11 mM) and ^{86}Rb as a tracer for potassium (total K^+ influx). K^+-influx assays were also performed in the presence of 0.1 mM ouabain (ouabain-resistant K^+ influx) and 0.1 mM ouabain plus 2 mM furosemide (ouabain-furosemide resistant K^+ influx).

Aliquots were withdrawn at time intervals and added to tubes containing 0.5 ml dibutylphthalate and centrifuged 1 min at $13000 \times g$. The supernatant was removed by aspiration and the walls of the tube thoroughly washed with isotonic NaCl to eliminate contaminating radioactivity. Then, after removal of the dibutylphthalate, the cells were precipitated by adding 0.6 ml of 5% trichloroacetic acid. After centrifugation, 0.5 ml of supernatant were removed and the radioactivity determined.

Red cell Li^+, Na^+ and K^+ concentrations were determined from $HClO_4$ lysates by absorption spectrophotometry (Li) or flame photometry (Eppendorf spectrophotometer).

All values were expressed as means ± S.D.

Fig. 1 shows a typical experiment of Na^+/Li^+ countertransport performed with horse and human red cells. The transport activities (lithium efflux) measured in this experiment for horse erythrocytes are 0.03 mmol (liter of red cells · h)$^{-1}$ in sodium free medium and 1.99 mmol · (liter of red cells · h)$^{-1}$ in sodium medium. The corresponding activities for a sample of human cells are 0.17 and 0.57 mmol · (liter of red cells · h)$^{-1}$. Results obtained in this type of experiment for six different samples of horse erythrocytes yield the following values of Li^+ efflux (mmol · (l cell · h)$^{-1}$: sodium medium, 2.21 ± 0.27 and Na^+-free medium, 0.10 ± 0.10. (The Li^+ concentration in these cells at the beginning of the experiment was 4.82 ± 0.28 ($n = 4$)). The average Na^+-dependent Li^+ efflux measured in horse erythrocytes (2.12 ± 0.27) is shown to be 8.8-times higher than the flux for the countertransport activity in human red cells of normal individuals, reported by Canessa et al. (0.24 ± 0.02) [12], and 5.9-times the countertransport activity of human cells as measured in our laboratory (0.36 ± 0.037).

A difference was also noticed in the ability of the red cells to accumulate lithium during the loading period. The lithium concentration after 3 h of incubation in 150 mM LiCl at 37°C was 4.82 ± 0.28 ($n = 4$) and 9.0 mM ± 1.29 ($n = 3$) in horse and human erythrocytes, respectively. Interestingly, less lithium was taken up by the horse cells which exhibit the higher Na^+/Li^+ countertransport activity. This suggests that the lithium which enters the cell under these experimental conditions, does so by means of transport systems other than the Na^+/Li^+ countertransport system, for example the Na^+/K^+ pump.

Fig. 2 shows determinations of unidirectional potassium influx in horse and human erythrocytes using ^{86}RB as a tracer. Measurements were done under three different conditions, as indicated above. This transport assay provides information about K movements that occur both through the Na^+ pump (ouabain sensitive flux), and the Na^+/K^+ cotransport system (furosemide-sensitive and ouabain-insensitive flux). Additional results obtained in this kind of experiment for six samples of horse erythrocytes are as follows (mmol · (l cell · h)$^{-1}$: Na^+ pump, 0.39 ± 0.12; Na^+/K^+ cotransport, -0.01 ± 0.02 and ouabain-furosemide resistant K^+ flux, 0.16 ± 0.1. Identical experiments performed with human erythrocytes ($n = 4$) yielded values of 1.32 ± 0.31 mmol · (l cell · h)$^{-1}$ for the K^+ movement through the sodium pump, 0.38 ± 0.19 for the Na^+/K^+ cotransport and 0.08 ± 0.05 for the 'passive flux.' Thus both the Na^+ pump activity and the cotransport activity seem reduced in horse erythrocytes, the pump

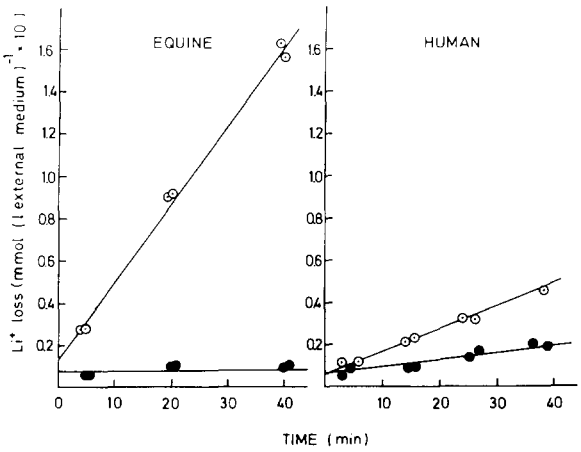

Fig. 1. Lithium efflux in horse and human erythrocytes. Lithium fluxes were determined in Na medium (○) and magnesium medium (●). The initial internal lithium concentrations were 4.8 and 9.4 mM in horse and human erythrocytes, respectively.

by a factor of 3.4-times while the Na^+/K^+ cotransport is undetectable under the present conditions.

The evidence summarized above therefore, shows a striking different pattern for the Na and K transport systems in horse erythrocytes when compared to those observed in human cells [1–8]. This observation has two important implications.

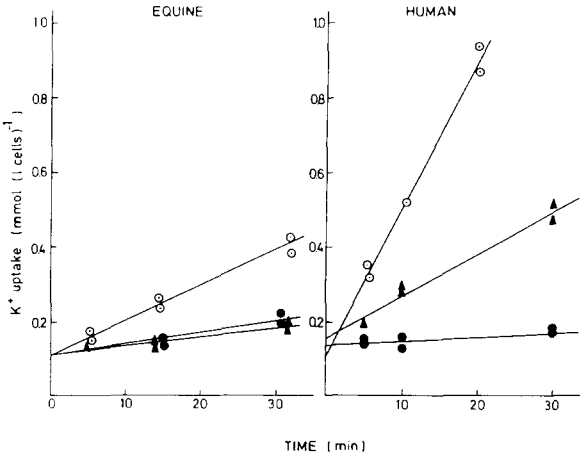

Fig. 2. Potassium uptake in horse and human erythrocytes. ^{86}Rb was used as a tracer to follow K^+ fluxes. The actual Rb^+ concentration was negligible and uptake was calculated on the basis of K^+ concentration. The uptake of ^{86}Rb was determined in the absence of inhibitors (○) and in the presence of 10^{-4} M ouabain (▲) or 10^{-4} M ouabain $+2\cdot10^{-3}$ M furosemide (●). The initial external potassium concentration was 4 mM.

TABLE I

INTRACELLULAR AND EXTRACELLULAR ELECTROLYTE CONCENTRATIONS IN HORSE ERYTHROCYTES

Values presented are means ± S.D. of nine different samples.

Electrolyte concentration (mM)			
Plasma		Cells	
Na^+	K^+	Na^+	K^+
137.0 ± 1.58	3.9 ± 0.14	10.4 ± 1.79	120 ± 11.1

First, these results confirm that the two ouabain-resistant systems express themselves independently, the Na^+-Li^+ exchange system being considerably more active than in human red cells while the Na^+/K^+ cotransport activity cannot be measured under our assay conditions. Second, this finding offers a new experimental model system where to test the relative contribution of the various Na^+ and K^+ permeabilities to the maintenance of the ionic gradients across the cell membrane. It is quite remarkable that the same intracellular Na^+ levels exist in horse and human erythrocytes (Table I) considering the difference in the activity of the transporters involved. It would also be important to investigate how the horse erythrocytes can cope with volume changes and whether the lack of Na^+/K^+ cotransport activity does affect the viability of the cells under these conditions. Finally, it is interesting to note that the typical kaliuretic effect of furosemide was not observed in horses when the drug was administered either intravenously or by stomach tube [13].

We thank Ms. Rosario Flores S. for her excellent technical assistance. This study was supported by grants from CONICYT (Fondo Nacional) and the University of Chile (DIB).

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