Biochimica et Biophysica Acta 899 (1987) 311–312
Elsevier

BBA 70506

BBA Report

Further evidence for coupling of sodium and proton movements in dog red blood cells

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(Received 26 January 1987)

Key words: Sodium ion transport; Exchange, Na^+–H^+; Tributyltin; cell pH; (Dog erythrocyte)

Using 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS) and tributyltin the sodium transport pathway activated by shrinkage in dog red blood cells is shown to behave as expected for an electroneutral Na^+/H^+ exchanger. When the driving forces for sodium and protons are equal, flow through the pathway stops. Amiloride inhibits the shrinkage-induced Na^+/H^+ exchange.

An electroneutral Na^+/H^+ exchanger is activated in dog red blood cells when they are shrunken [1], when their internal pH is reduced [2], or when their cytoplasmic lithium content is raised [2]. Movements of sodium and protons through this pathway are inhibited by amiloride, by quinidine, and by external protons and lithium ions [2,3]. Activation of the exchanger by shrinkage can be inhibited by substituting nitrate or thiocyanate for chloride, although chloride is not involved in the process of Na^+/H^+ counterflow [4].

Herein we report the effects of tributyltin on sodium movements through the Na^+/H^+ exchanger of dog red blood cells. Tributyltin is of interest in this system because it functions in red cells as a Cl^-/OH^- exchanger [5] and is therefore able to shunt out the proton gradient that is generated by the Na^+/H^+ countertransport in DIDS-treated red cells. With tributyltin we get an electrosilent pH-equilibrating exchange diffusion of hydroxyl ions without a net transfer of protons and of electrical current which would have been the case by using the proton carrier carbonylcyanide *m*-chlorophenylhydrazone (CCCP).

For a neutral Na^+/H^+ exchanger the driving force for sodium movement would equal the difference between the chemical potential gradients for Na^+ and H^+

$$[\ln(Na_i^+/Na_o^+) - \ln(H_i^+/H_o^+)]\ RT/F$$

where Na_i^+ and Na_o^+ and H_i^+ and H_o^+ refer to the chemical activities of sodium and protons in the intracellular (i) and outside (o) media, and R, T, and F have their usual meanings [6].

Thus, if $Na_i^+/Na_o^+ = H_i^+/H_o^+$, there will be no driving force for sodium movement. If $Na_i^+/Na_o^+ > H_i^+/H_o^+$, sodium will move outward, and if $Na_i^+/Na_o^+ < H_i^+/H_o^+$, sodium will move inward through an electroneutral Na^+/H^+ exchanger.

For the experiments was used freshly drawn

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heparinized blood from dogs. The erythrocytes were washed, DIDS-treated, and analyzed as described in Ref. 7. The chloride exchange system in dog erythrocytes was reduced to 0.5% of the control values when $1.1 \cdot 10^6$ DIDS molecules were bound per cell as in human red blood cells. A further reduction of the chloride exchange to about 0.005% could be obtained by having 50 μm DIDS in the media. To prevent shunting of protons via the Jacobs Stewart cycle [8] CO_2-free solutions were used and the experiments were performed in the presence of the carbonic anhydrase inhibitor ethoxyzolamide.

Each of the three experiments in Fig. 1 begins with the injection of 1 volume of dog red cells into 4 vols. of unbuffered, CO_2-free 240 mM KCl solution.

Because sodium in the cytoplasm is high, Na_i^+/Na_o^+ is high. Because the solution is hypertonic, the cells shrink, and the putative Na^+/H^+ exchanger is activated. During the first phase of each incubation sodium leaves the cells in exchange for protons. Because the cells have been pretreated with DIDS, equilibration of pH across the membrane is greatly retarded, and the medium promptly becomes alkaline, reaching a maximum pH after 4–5 min. The driving force for sodium efflux is diminished as H_o^+ drops and H_i^+/H_o^+ approaches Na_i^+/Na_o^+.

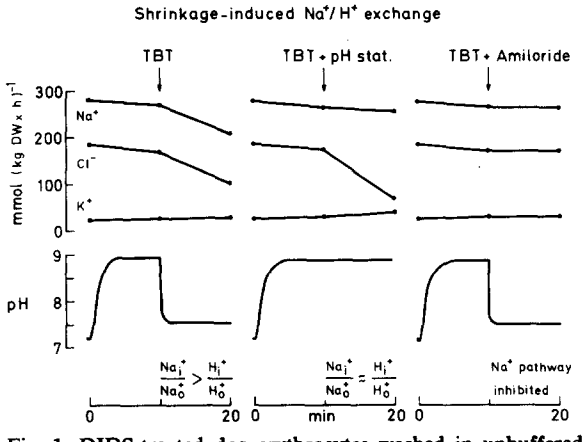

Fig. 1. DIDS-treated dog erythrocytes washed in unbuffered CO_2-free isotonic KCl were at time zero injected into a hypertonic medium containing 240 mM KCl, 5 mM glucose, 0.5 mM EDTA, 0.05 mM DIDS, and 0.05 mM ethoxyzolamide; temperature 38° C; hematocrit 20 vol%. At the time indicated tributyltin (TBT) and amiloride were added to give the following concentrations in the cell suspension: tributyltin 5 μM, amiloride 0.25 mM. As titrant was used 50 mM KOH.

In the left panel tributyltin is added at the 10-min point, and sodium efflux is promptly stimulated. This is because tributyltin acts as a Cl^-/OH^- exchanger and rapidly dissipates the pH gradient that had been established during the first part of the experiment. As H_o^+ is suddenly increased, Na_i^+/Na_o^+ exceedes H_i^+/H_o^+ and this acts as a driving force for outward sodium countertransport. Chloride moves easily outward in exchange for hydroxyl ions, which are transported inward by tributyltin.

In the middle panel tributyltin is again added at the 10-min point, but no sodium efflux is seen. This is because a pH stat is activated coincident with the addition of tributyltin, and H_o^+ is kept at its low plateau level. Since H_i^+/H_o^+ is maintained at a value near Na_i^+/Na_o^+, the driving force for sodium is nil. Chloride is lost from the cells because of tributyltin-mediated Cl^-/OH^- exchange, OH^- being added to the system by the pH stat.

In the right panel tributyltin is added at the 10-min point, again without an increase in sodium efflux, although the expected fall in external pH provides a driving force equal to that of the experiment in the left panel. This result is explained by the simultaneous addition of amiloride, a blocker of Na^+/H^+ exchange.

These experiments demonstrate that amiloride-sensitive sodium movements in dog red blood cells are tightly coupled to the proton concentration on the *trans* side of the membrane. In addition, these studies show that neither of two very useful compounds, DIDS or tributyltin, inhibit the Na^+/H^+ exchanger in dog red blood cells. In trout red blood cells alkyl tin derivatives inhibit Na^+/H^+ exchange [9].

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