Studies on the Lithium Transport across the Red Cell Membrane

III. Factors Contributing to the Intraindividual Variability of the in vitro Li⁺ Distribution across the Human Red Cell Membrane

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Summary. 1. Extracellular potassium, bicarbonate, ouabain, dipyridamole and the Na⁺ distribution between red cells and plasma influence Li⁺ transport across the human red cell membrane. The significance of these parameters for the intraindividual variability of the steady-state ratio of external to internal Li⁺ was studied in vitro.

2. Elevation of external K⁺ in the physiological concentration range increases the steady-state distribution ratio Li⁺/Li⁻ indirectly by increasing the ratio Na⁺/Na⁻ through activation of the Na⁺-K⁺ pump, and directly by inhibiting ouabain-sensitive Li⁺ uptake.

3. A rise in bicarbonate concentration decreases the Li⁺ ratio directly by accelerating Li⁺ uptake through a leak, and indirectly by increasing the Na⁺ leak, thus reducing the Na⁺ ratio.

4. Dipyridamole blocks both bicarbonate effects.

5. Ouabain decreases the Na⁺ ratio and inhibits Li⁺ uptake by the Na⁺-K⁺ pump, thereby exerting two opposite effects on the Li⁺ distribution ratio.

6. The results confirm the previous observation that the steady-state Li⁺ distribution depends strongly on the Na⁺ distribution ratio, i.e., the driving force for Na⁺-dependent Li⁺ uphill countertransport. It is concluded that the Na⁺ distribution between red cells and plasma and the concentrations of K⁺ and bicarbonate in plasma need to be considered as factors influencing the in vivo Li⁺ distribution. However, the considerable interindividual differences of Li⁺ distribution cannot be ascribed to variations in these parameters.

Key words: Lithium-transport - Erythrocytes - Potassium - Sodium ratio - Ouabain - Bicarbonate - Dipyridamole.

INTRODUCTION

Considerable interindividual differences in the steady-state distribution of Li⁺ between red cells and plasma have been observed during therapy of affective diseases with lithium salts [12]. According to several authors, the steady-state Li⁺ distribution ratio remains fairly constant within any one individual [4, 9, 13], but intra-individual fluctuations of the ratio have also been reported [5, 15].

Previous studies on Li⁺ transfer across the red cell membrane demonstrated that three mechanisms can participate in Li⁺ transport: the ouabain-insensitive, Na⁺-dependent Li⁺ countertransport system which is largely responsible for the low red cell Li⁺ concentration in vivo [3, 8, 10], K⁺- and ouabain-sensitive Li⁺ uptake by the Na⁺-K⁺ pump [2], and bicarbonate-stimulated diffusion through a leak [2, 16]. It can be inferred from these results that changes in the transmembrane Na⁺ distribution and the concentrations of K⁺ and bicarbonate in the plasma might alter the in vivo Li⁺ distribution. The influence of these parameters on the steady-state Li⁺ distribution across the red cell membrane was therefore analyzed in vitro. Additionally, the effects of ouabain and dipyridamole were studied.

METHODS

Standard Incubation System. The studies were performed with red cells of a single donor (U.L.). Blood was drawn into heparinized syringes and the buffy coat was removed after centrifugation. The erythrocytes were washed once and loaded with Li⁺ (15 min pre-incubation at 37°C and pH 7.4) in a medium containing 100 mM LiCl, 50 mM NaCl, 5 mM glucose, 1 mM CaCl₂ and 0.5 mM MgCl₂. The loaded cells were washed three times in the prospective incubation medium (0°C) and then suspended at an hematocrit of 1–2% in media containing 2 mM LiCl, 10 mM glucose, 1 mM inorganic phosphate, 1 mM CaCl₂, 0.5 mM MgCl₂ and NaCl and KCl varying between 0 and 150 mM. Isotonicity was maintained with choline chloride. The initial distribution ratio Li⁺/Li⁻ thus obtained
was about 2 (subscripts e and i: extracellular and intracellular, respectively). Penicillin and streptomycin were added at final concentrations of 0.2 and 0.1 mg/ml, respectively. The suspensions were incubated for 27 h at pH 7.4 and 37°C with agitation in polyethylene flasks. The pH was repeatedly adjusted with tris-(hydroxymethyl)-aminomethane and not allowed to decrease below 7.25. In some experiments chloride was replaced by bicarbonate to yield extracellular concentrations of 23 or 130 mM bicarbonate. These red cell suspensions (hematocrit 8–10%) were equilibrated with gas mixtures containing 5.6% and 40% CO₂, respectively, prepared from air and CO₂ by Wösthoff pumps. The pH value thus obtained ranged between 7.35 and 7.40.

Analytical Procedures. Samples of the cell suspension were taken after 3, 6, 22 and 27 h of incubation, cooled in an ice bath and the supernatants removed after centrifugation (0°C). The cells were then washed three times in a 10-fold volume of isotonic choline chloride at 0°C and finally centrifuged for 4 min at 9500 x g. Measurements of Li⁺, Na⁺ and K⁺ contents of packed cells and of supernatants were carried out after suitable dilution with 6% 1-butanol (atomic absorption spectrophotometer Perkin Elmer 400). The distribution ratios of Li⁺ and Na⁺ refer to amount per volume of medium and red cells, respectively.

RESULTS

Effects of External Na⁺, K⁺ and Ouabain

The transmembrane Na⁺ gradient was varied at three different external K⁺ concentrations by exchanging external Na⁺ with choline⁺, as well as by adding ouabain. The steady-state ratios of external to internal Li⁺ and Na⁺ established after 27 h of incubation of Li⁺-preloaded cells in the standard incubation system are summarized in Figure 1.

In the absence of ouabain, the Li⁺ and the Na⁺ ratios increased with rising external Na⁺ concentration at all K⁺ levels. The Na⁺ effect was more pronounced at the higher K⁺ concentrations (solid lines in Fig. 1). At any given external Na⁺ concentration, the increase in the Li⁺ ratio due to K⁺ slightly exceeded the increase in the Na⁺ ratio. It is to be noted that the steady-state Li⁺ distribution ratio Li⁺/Li⁺ was 0.5 in the absence of added Na⁺ and K⁺, i.e., an inverse ratio Li⁺/Li⁺ of 2 had been established under these conditions (Fig. 1, lower left hand panel). This is due to the action of the Na⁺-K⁺ pump which is capable of transporting Li⁺ into the cells against an electrochemical gradient, provided that this transport property is not inhibited by ouabain, external K⁺ or external Na⁺ (cf. [2]).

Ouabain caused a reduction of the distribution ratios of both Li⁺ and Na⁺ at 5 and 25 mM external K⁺. The Na⁺ ratios decreased to a greater extent than the Li⁺ ratios. At external K⁺ concentrations below 0.5 mM, the Li⁺ ratio was elevated by ouabain, whereas the Na⁺ ratio was slightly diminished. In the presence of ouabain, the Na⁺ and Li⁺ ratios were

![Fig. 1](image-url) Fig. 1. Effects of extracellular Na⁺ and of ouabain on the Li⁺ and Na⁺ distributions between red cells and medium at three different external K⁺ concentrations. The distribution ratios were determined after 27 h of incubation in the standard incubation system (see Methods). External Na⁺ and K⁺ were replaced by choline⁺ (pH 7.4, 37°C). (—) controls; (○——○) 0.1 mM ouabain.