J. Physiol. Biochem., 52 (2), 77-82, 1996
Revista española de Fisiología

Human red cells from prenatal stages of hemopoiesis. Lithium flux components

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(Received on October 17, 1995)

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Red cells from umbilical cord with increased lithium content were submitted to different experimental conditions in order to study lithium flux components. There appeared three components: First, an ouabain-sensitive component, related to Na^+ replacement with Li^+ in the primary active Na^+/K^+ transport system. The magnitude of this fraction is greater than in adults' red cells. Second, an outside sodium-dependent Li^+ efflux fraction, corresponding to the Li^+/Na^+ countertransport system with V_{max} and K_m values of 0.1 (mmol/l cells • h) and 2.58 (mmol/l), respectively. The Na^+_o-affinity for lithium efflux in this system is greater in neonatal than in adults' red cells. Third, a leak fraction with an equal value to that reported in adults' red cells. Furthermore, the possible non-existence of a bumetanide-sensitive lithium flux fraction was shown in neonatal red cells

Key words: Neonatal red cells, Lithium transport.

The erythrocyte has an established reputation as a convenient and readily accesible cell system for the characterization of membrane transport processes.

Differences in some cationic transport mechanisms have been reported to occur between neonatal (nRC, product of prenatal hemopoietic stages) and adult's red cells (aRC) (2, 3, 5, 13-15).

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Cation transport systems can translocate, some "foreign" ones, in addition to those naturally involved in a determined mechanism.

Lithium ions can be transported across the plasma membrane by several systems: primary active (Na^+/K^+ pump), Na^+/H^+ countertransport, Na^+/Na^+ exchange and inorganic anion exchange (6, 8, 10, 16).

Na^+/Li^+ countertransport (CTT) presents physiopathological concern as CTT

activity is associated with the risk of essential hypertension and diabetic nephropathy (12, 17, 19).

The following study describes lithium flux components in nRC to determine transport characteristics for this "foreign" cation (Li^+) in red cells from prenatal stages of hemopoietic development (23). Lithium was selected for the measurement of some of the mentioned transport systems because it provides a reliable estimation of sodium fluxes (8, 10).

Materials and Methods

Source and general handling of cells.– Human blood from normal adults and from the umbilical cord of normal deliveries were used. The general methodology employed for the preparation of cells has been described in detail previously (20). The cells were resuspended in isotonic buffered (Tris-MOPS) solution (Na^+ as principal cation), centrifuged and resuspended again in the same medium after discarding the supernatant (washed cells).

Incubation procedures.– Intracellular Li^+ was adjusted to desired concentrations by incubation with Li_2CO_3 during variable time intervals (10).

Fresh red cells were washed three times with 10 volume of an isotonic buffered solution containing Li_2CO_3 (20 mmol/l), and bubbled with carbon dioxide for variable time intervals at 37 °C in a shaker-bath. Then the cells were suspended in the appropriate Li^+ free medium of the following composition (mmol/l): Tris-MOPS, 10; pH 7.4, $CaCl_2$, 0.1; KCl, 0.1; NaCl plus $MgCl_2$, 140 (isoosmotic replacement of NaCl by $MgCl_2$); glucose, 10; and ouabain, 0.1. They were then centrifuged, washed and resuspended in the same medium. The cells were washed four times in the same way and centrifuged finally for 60 s.

Li^+-flux determination.– To study Li^+ efflux the washed cells were finally resuspended in the medium described above to a final hematocrit of 3-5 % and incubated at 37 °C for 30 min. At 4-6 time intervals, about 2 ml aliquots were removed and centrifuged at 10000 *g* for 1 min in a Beckman microfuge and supernatants were removed for determination of Li^+.

A time interval of linear increase of lithium concentration in the medium was determined. $Li^+_{(o)}/Li^+_{(i)}$ ratios at the end of efflux periods were approximately 1/200. The efflux was expressed in mmol Li^+/l initial number of cells (correction to initial volume). All procedures were carried out in duplicate. Lithium concentration in cell lysates or in supernatants was determined by atomic absorption spectrophotometry in a Perkin Elmer spectrophotometer model 2380.

Determination of cellular volume.— Samples were taken and processed in a manner previously described (21). Briefly, after centrifugation of the cell suspension and aspiration of enough supernatant to increase the hematocrit to = 50 %, hemoglobin (Hb) was determined by the cyanmethemoglobin method. Samples from each group of packed cells were appropriately diluted into 5 ml of Drabkin's solution and optical density measured at 540 nm on a Beckman spectrophotometer. The hematocrit of packed cells were measured in microcapillary tubes (microcapillary centrifuge). The cellular volume was expressed as relative cellular volume (rcv) and was calculated from the ratio of Hb and hematocrit measurements at different times related to the same ratio of fresh (unincubated) cells.

Determination of cell lysis.– Optical density of supernatant of incubated suspensions was expressed as a fraction of the optical density of cell lysates (100 %). A Beckman DU 2 spectrophotometer, (414 nm) was used.

Reagents.– Ouabain, Tris-MOPS were obtained from Sigma. Drabkin's reagent and haemoglobin standards from Wiener Lab. (Argentina). All the other drugs used were also of analytical grade.

Results

Cellular lithium content.– Cellular suspensions (at low hematocrit) were incubated in 20 mmol/l Li_2CO_3 medium, with aliquots removed at different times for analysis of cellular lithium content. Figure 1 shows a proportionality between incubation time and cellular lithium levels; the insert, shows that the relative cellular volume varied, during the efflux period, within its determination error.

Lithium efflux components.– In 140 mmol/l Na^+ media and at cellular lithium levels over 5 mmol/l cells, the efflux in the absence minus that in the presence of 0.1 mmol/l ouabain corresponds to the lithium component mediated by the sodium/potassium pump. It amounted to 0.07 ± 0.009 and 0.03 ± 0.01 (mmol/l cells • h) for nRC and aRC, respectively ($p < 0.05$). In the presence of 0.1 mmol/l ouabain the efflux measured at 140 mmol/l Na^+ minus that obtained in the absence of Na^+ measures the Na^+/Li^+ countertransport component (0.11 ± 0.006 and 0.07 ± 0.01 mmol/l cells • h for nRC and aRC, respectively). The efflux component determined in media 0.1 mmol/l ouabain and in the absence of sodium measures the leak (0.10 ± 0.004 and 0.09 ± 0.007 mmol/l cells • h for nRC and aRC, respectively). A bumetanide-sensitive component was individualized in aRC, that amounted to 0.12 ± 0.02 but not in nRC (0.001 ± 0.04 mmol/l cells • h).

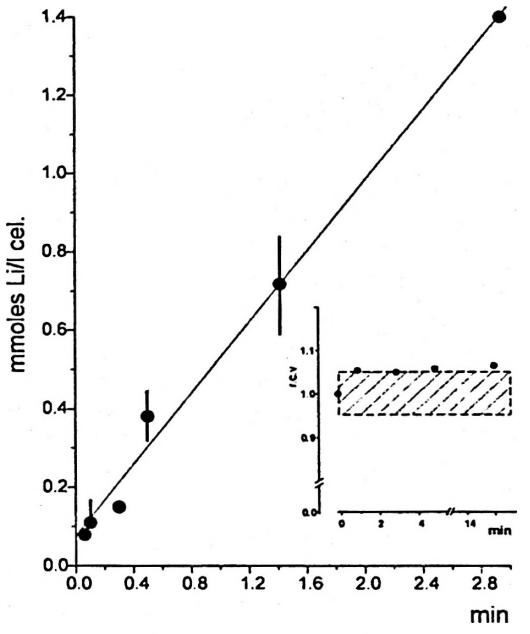

Fig. 1. *Neonatal red cells. Cellular lithium content as a function of time. Incubation with Li_2CO_3.* Data are presented as media ± SM from determinations realized by duplicate in samples from 5-7 different individuals. The equation: cell lithium = a + bt fitted the experimental data [a (mmol/l cells) = 0.52 ± 0.20 and b (min^{-1}) = 0.45 ± 0.021]. *Insert:* Neonatal red cells, volume during Li^+ efflux determination. Hatched area indicates SEM relative cellular volume (r.c.v.) estimation (± 0.039). This error was calculated according to a function of alleatory variables; r.c.v. during the Li^+ efflux in cells previously treated with Li_2CO_3 (cellular Li^+ content: 5-7 mmol/l cells). Data presented correspond to the mean of samples from 5-7 different individuals. The index of dispersion was lesser than 10 % of the corresponding mean value.

Sodium/lithium countertransport. Kinetic parameters for the "foreign" (Li^+) and "natural" (Na^+) substrates.– The analysis of lithium flux through this system as function of cellular Li^+ content and, at saturating $Li^+_{(i)}$, as a function of

$Na^+_{(o)}$ showed that a Michaelis-Menten mechanism is operative. The lithium affinity was, in nRC, similar to that in aRC. However, the apparent affinity for external Na^+ [Km $(Na^+)_{(o)}$] was significantly lower, in nRC as compared to aRC. No significative difference was shown when comparing the Vmax values for both cell types (table I).

Table I. *Na^+/Li^+ CTT kinetic parameters.* Determinations: i) as a function of cellular lithium content (0.1 - 5 mmol/l cells) at a saturating point outside sodium concentration (140 mmol/l) for the system, and ii) as a function of extracellular sodium concentration (1-140 mmol/l, isoosmotic replacement by $MgCl_2$) at saturating cellular lithium contents (5 mmol/l cells) for the system. Upper value corresponds to experimental condition (i); lower value corresponds to experimental condition (ii). Fitting of experimental data: hyperbolic equation. Mean ± SE error for determinations realized in samples from 6-8 different individuals are presented.

Cells		Km	Vmax
nRC	i)	0.6 ± 0.2	0.13 ± 0.01
	ii)	2.58 ± 0.5	0.10 ± 0.01
aRC	i)	0.5 ± 0.2	0.13 ± 0.006
	ii)	10.78 ± 2.9	0.13 ± 0.01

Discussion

Transport mechanism differences between nRC and aRC are special issues of distinct structural-functional features reported for these cells (2, 3, 5, 9, 13-15, 23).

Ionic transport mechanisms can translocate "natural" as well as "foreign" ions. Lithium ion, a "foreign" ligand for a variety of transport mechanisms may be used as a marker for the measurement of their flux values. Some transport mechanisms that accept lithium are differently expressed in diseases with genetic basis (12, 17-19) as well as during ontogenic development. A reduced lithium flux related to sodium-lithium countertransport (1) but not to sodium/potassium cotransport (4) has been reported at birth in red cells with reference to similar cells from adults. Developmental changes in a transport system can involve the maximum flux and/or the affinity for the transported ligands (22). The diversity of lithium transport pathways makes it necessary to select experimental conditions in which no other lithium transport pathways, distinct from that in study, could be operative.

In the present paper, conditions were selected to satisfy those referred above studying lithium efflux in neonatal and adults' red cells. Leak flux values did not differ between either cell type. Other components differed either in the flux value (ouabain sensitive) or in the affinity for the transported ions. In $Na^+_{(o)}$ dependent Li^+ efflux (Na^+ /Li^+ countertransport) a significative decrease in the affinity for the "natural" ligand ($Na^+_{(o)}$) is developed on switching from pre to postnatal stages of hemopoiesis. The affinity for the "foreign" ligand in this system however did not differ for cells from both stages of hemopoietic development.

It is to be noted that the sum of lithium flux components amounted, in nRC, to the total measured flux (0.28 ± 0.02 mmol/l cells • h). This suggests that other flux components (e.g. bumetanide-sensitive) would be absent (20, 21). This is not the case for aRC where the difference between the total (0.30 ± 0.08) and the addition of the fractions measured (excluding the bumetanide-sensitive) amounted to 0.11 mmol/l cells • h. This value corresponds to the bumetanide-sensitive flux component as has been reported (11). The absence of a bumetanide-sensitive lithium flux component in nRC (although a bumetanide-sensitive potassi-

um flux has been reported for these cells) suggests that the $Na^+/K^+/2\ Cl^-$ cotransport system (in nRC) would not accept lithium with similar requirements from those referred for adults' red cells (21).

Acknowledgements

The authors would like to thank Mr D. Taborda for technical assistance. The work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

J. L. CORCHS, G. MUJICA y R. E. SERRANI. *Eritrocitos humanos de etapas prenatales de hematopoiesis. Componentes del flujo de litio.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (2), 77-82, 1996.

Se estudian los componentes del flujo de litio en eritrocitos de cordón umbilical cargados con el cation, en diferentes condiciones experimentales. Se demuestra la existencia de los siguientes componentes: 1) sensible a ouabaina, relacionado con la sustitución de Na^+ por Li^+ en el sistema de transporte activo primario Na^+/K^+. La magnitud de esta fracción es mayor que en eritrocitos de adultos; 2) un componente de salida de Li^+ dependiente de Na^+ externo, relacionado el sistema de contratransporte Li^+/Na^+, V_{max} = 0.1 mmol/l cel • h K_m = 2.58 mmol/l. La afinidad por el sodio de este sistema es mayor en eritrocitos neonatales que en los de adultos; y 3) una fracción de "pérdida" de un valor igual al referido para eritrocitos de adultos. No se observa la existencia de un componente de flujo de salida de Li^+ sensible a la bumetanida en eritrocitos neonatales.

Palabras claves: Eritrocitos neonatales, Transporte de Li^+.

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