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Li/Na Exchange and Li Active Transport in Human Lymphoid Cells U937 Cultured in Lithium Media

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Abstract—Lithium transport across the cell membrane is interesting in the light of general cell physiology and because of its alteration during numerous human diseases. The mechanism of Li^+ transfer has been studied mainly in erythrocytes with a slow kinetics of ion exchange and therefore under the unbalanced ion distribution. Proliferating cultured cells with a rapid ion exchange have not been used practically in study of Li^+ transport. In the present paper, the kinetics of Li^+ uptake and exit, as well as its balanced distribution across the plasma membrane of U937 cells, were studied at minimal external Li^+ concentrations and after the whole replacement of external Na^+ for Li^+. It is found that a balanced Li^+ distribution attained at a high rate similar to that for Na^+ and Cl^- and that Li^+/Na^+ discrimination under balanced ion distribution at 1–10 mM external Li^+ stays on 3 and drops to 1 following Na, K-ATPase pump blocking by ouabain. About 80% of the total Li^+ flux across the plasma membrane under the balanced Li^+ distribution at 5 mM external Li^+ accounts for the equivalent Li^+/Li^+ exchange. The majority of the Li^+ flux into the cell down the electrochemical gradient is a flux through channels and its small part may account for the NC and NKCC cotransport influxes. The downhill Li^+ influxes are balanced by the uphill Li^+ efflux involved in Li^+/Na^+ exchange. The Na^+ flux involved in the countertransport with the Li^+ accounts for about 0.5% of the total Na^+ flux across the plasma membrane. The study of Li^+ transport is an important approach to understanding the mechanism of the equivalent $Li^+/Li^+/Na^+/Na^+$ exchange, because no blockers of this mode of ion transfer are known and it cannot be revealed by electrophysiological methods. Cells cultured in the medium where Na^+ is replaced for Li^+ are recommended as an object for studying cells without the Na,K-ATPase pump and with very low intracellular Na^+ and K^+ concentration.

Keywords: Li^+/Na^+ exchange, lithium, ion transport, U937 cells

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Of the monovalent ions, Li^+ is the closest analogue to Na^+. Lithium distribution between the cells and environment under natural conditions and Li^+ distribution between erythrocytes and blood plasma in patients systematically taking Li^+ is near to the distribution of sodium, but is drastically different from that of potassium, rubidium, and cesium. It has been shown that, under physiological conditions, the Na, K-ATPase pump is not involved in Li^+ efflux in human erythrocytes and other objects (Pandey et al., 1978; Ehrlich and Diamond, 1979). According to the current views, active Li^+ flux against the electrochemical gradient occurs due to its coupling with the sodium transport down the electrochemical gradient (Kemp et al., 2008). It aligns Li^+ and Na^+ gradients on the cell membrane. Coupling of lithium and sodium fluxes (Li^+/Na^+ exchange) may occur in various systems of sodium transport across the cell membrane (Lytle et al., 1998; Elmariah and Gunn, 2003). In cells of different tissue and species origin, various transporters play the principal role in coupled lithium and sodium distribution. The study of Li^+/Na^+ countertransport started more than 30 years ago, when it was found that its modification in erythrocytes was common for a number of widespread human pathologies, such as hypertension (Canessa et al., 1980; West et al., 1998; Semplicini et al., 2003), diabetes, nephropathy, and others (Zerbini et al., 2004). The interest in Li^+ is due to the fact that Li^+ is applied as a medicine in psychiatry (Lenox, Le Wang, 2003). For general cellular physiology, Li^+ is attracting attention as a Na^+ surrogate not pumped with the Na,K-ATPase pump.

Abbreviations: DMA—dimethylamiloride, NMDG-Cl—N-methyl-D-glucamine chloride, MgGluc—magnesium gluconate, AO—acridine orange, EB—ethidium bromide, NC—Na–Cl symporter, NKCC—Na–K–2Cl cotransporter, NHE—Na^+/H^+ exchanger.

Li^+/Na^+ countertransport studies using cultured cells usually concern transporter genomics. However, an important advantage of these cells over erythrocytes widely used for Li^+/Na^+ countertransport assay is a much more rapid equilibration of Li^+ and Na^+ between intra- and extracellular media. The balanced distribution of these ions can be easily obtained in a short-term experiment in vitro with cultured cells but not with erythrocytes.

The present study aimed at analyzing lithium transport in and out of the cells and its balanced distribution. U937 human histiocytic lymphoma cells were used as an object. The flux balance for three major monovalent ions, Na^+, K^+ and Cl^-, has been more fully studied in these cells than in other cultured animal cells (Mills and Tupper 1975; Tupper, 1975; Vereninov et al., 2007; Yurinskaya et al., 2011).

MATERIALS AND METHODS

The basic materials and methods used in this work have been described before (Vereninov et al., 2004, 2007; Yurinskaya et al., 2005, 2010, 2011a, 2011b). U937 human histiocytic lymphoma cells were obtained from the Russian Cell Culture Collection (cat. no. 160B2). The cells were cultured in RPMI 1640 medium (Biolot, Russia) with 10% fetal calf serum (HyClone, Standard, United States). Ouabain (0.01–0.1 mM), dimethylamiloride (DMA, 0.05 mM), bumetanide (0.05 mM), phloretin (0.03 mM), and metolazone (0.03 mM) (all from Sigma-Aldrich, Germany) were used for analysis of ion fluxes. Water content in cells was evaluated by cell buoyant density in the Percoll density gradient. Intracellular ion content was determined by flame emission with a Perkin-Elmer AA 306 spectrophotometer. The protein amount was measured by the Lowry method. Rate constants of ion exchange between the cells and medium were estimated by the rate of their influx or efflux. Li^+ efflux into the culture medium was estimated with 5 mM LiCl added into the culture medium; Na^+ and Cl^- fluxes were measured using $^{22}Na^+$ and $^{36}Cl^-$ (Isotope, Russia). To study ion efflux, 5 mM LiCl and 1 mM RbCl were added to the culture medium 20 h before the experiment and marker isotopes $^{22}Na^+$ and $^{36}Cl^-$ were added 1.5 h before the experiment. The cells were then rapidly washed from the medium marker ions by 96 mM $MgCl_2$ and placed into the RPMI medium without Li^+, Rb^+, $^{22}Na^+$ and $^{36}Cl^-$. The ion efflux rate was measured in standard RPMI medium, as well as in media without Na^+ (96 mM $MgCl_2$ or 140 mM NMDG-Cl + 5.5 mM RbCl) or beside Na^+ also Cl^- ions (96 mM magnesium gluconate) and pH adjusted to 7.4–7.6 with Tris-OH and MOPS. Rate constants of the ion exchange were calculated according to $y(t) = y_{t=\infty}(1 - \exp(-kt))$ for influx and $y(t) = y_0\exp(-kt)$ for efflux, where $y(t)$ is the content of the ion-label at time moment t (5, 10, 20 min) and $y_{t=\infty}$ and y_0 are the final and initial content of the corresponding ion.

The cells were stained with acridine orange (AO, 10 μg/mL) and ethidium bromide (10 μg/mL) mixture and visualized under a Leica TCS SL confocal microscope. Fluorescence was excited with 488 nm and registered at 500–550 and 600–700 nm. Cell visualization in the transmitted light was done with an Axiovert 200M microscope equipped with a Leica DFC 420 camera (Germany).

The results were statistically treated using Student's t-test. The difference was considered significant at $p < 0.05$.

RESULTS

Li^+ exchange rate in U937 cells under the balanced state. Measurements of influx and efflux of lithium-label show that Li^+ balanced distribution in U937 cells is established as rapidly as that for Na^+ and Cl^- (Fig. 1). The rate constant of Li^+ exchange between the cells and RPMI standard medium is 0.04–0.06 min^{-1} (Table 1, Fig. 2). Given this exchange rate, it is possible to consider that the incubation for 1.5 h is sufficient to reach the balanced Li^+ distribution. Cell cultivation for 24 h in the medium with 1–10 mM LiCl confirms this. The rate constants of Li^+ and Na^+ exchange in U937 cells are near to those reported for HeLa cells (Boardman et al., 1975). The rate constant of Li^+ exchange in human erythrocytes under physiological conditions is about 0.0035 min^{-1} (Ehrlich, Diamond, 1979).

Intracellular content and Li^+ exchange kinetics in U937 cells incubated in the medium with low Li^+ concentrations. Li^+ addition in the culture medium in concentration 3–5 mM for 12–24 h has no effect on U937 cell proliferation and morphology assayed under a light microscope (not shown). Altered morphology and slower growth were reported in cells cultivated with 10 mM Li^+ and higher and for 24 h and longer (Matthopoulos et al., 1995; Laurenz and Smith 1998). The study of Li^+ balanced distribution in U937 cells with its low concentration shows that, under an Li^+ external concentration of 1–10 mM, its intracellular concentration is 10–20% lower than in the culture medium (Table 1).

Li^+, like sodium, is not accumulated in cells as cations should be if their distribution depends only on the electric potential difference between the cytoplasm and the environment. At the same time, the coefficients of Li^+ and Na^+ distribution between the cytoplasm and medium in U937 cells differ by three times if the Na, K-ATPase pump has not been blocked with ouabain. The Li^+/Na^+ discrimination remains constant under external Li^+ concentration within the range 1–10 mM. We can conclude, therefore, that Li^+

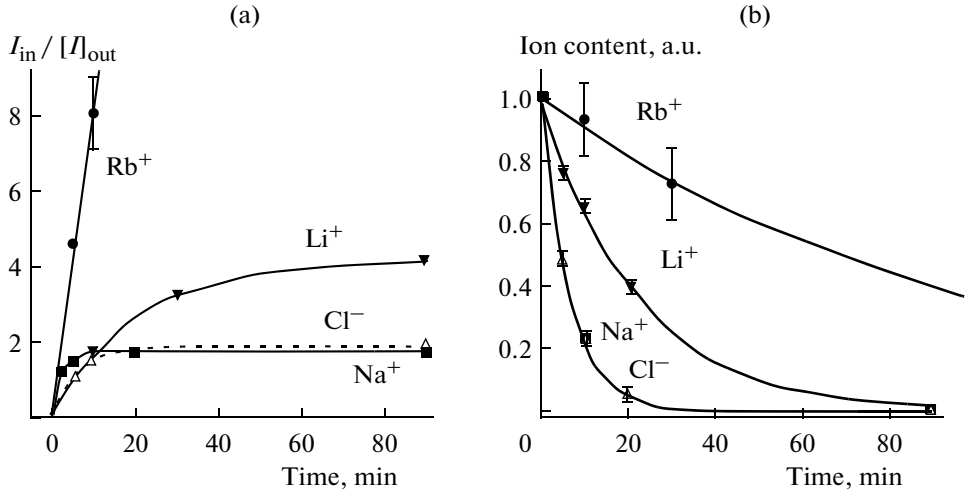

Fig. 1. Kinetics of Li^+, $^{22}Na^+$, Rb^+, and $^{36}Cl^-$ (a) gain and (b) release in U937 cells in RPMI medium.
(a) $I_{in}/[I]_{out}$ is the ratio of the ion I content (Rb^+, Li^+, Na^+, Cl^-, μmole per 1 g protein) to its concentration in the medium, mM. At $t = 0$, LiCl (5mM), RbCl (2.5 mM), and isotopes were added to the medium; (b) cells preincubated in RPMI medium supplemented with 5 mM LiCl and 1 mM RbCl (20 h) and isotopes $^{36}Cl^-$ and $^{22}Na^+$ (1.5 h) were washed from this medium with $MgCl_2$ solution and placed into RPMI medium without Li^+, Rb^+, $^{36}Cl^-$, and $^{22}Na^+$ ($t = 0$). Each point on the figure is a mean value and standard error from three or four experiments with two or three parallels.

distribution is tightly coupled with Na^+ distribution, which is constant under the variation of low LiCl concentration. The threefold difference in Li^+ and Na^+ distribution coefficients is close to the ratio of the rate constants for Li^+ and Na^+ efflux (about 2.5 times).Therefore, Li^+/Na^+ discrimination in U937 cells is determined by the Na, K-ATPase pump rather than cell-entrance pathways for Li^+ and Na^+. The coefficients of Li^+ and Na^+ distribution become similar if the pump is switched off.

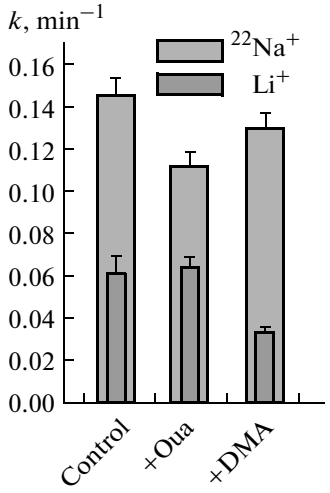

Fig. 2. Rate constants k_{Na} and k_{Li} in U937 cells calculated by exit of $^{22}Na^+$ and Li^+ into RPMI medium with and without inhibitors of the Na, K pump (ouabain) and Na^+/H^+ exchanger (DMA).
The cells preincubated for 1.5 h in RPMI medium with $^{22}Na^+$ and 5 mM LiCl, were transferred for 10 min into a medium free of $^{22}Na^+$ and Li^+ with or without 0.1 mM ouabain or 0.05 mM DMA. Rate constants were calculated by the formula $k = -(1/t)\ln y_t/y_0$, where y_t is intracellular content of $^{22}Na^+$ or Li^+ at time $t = 10$ min and y_0 is initial content of the corresponding ion. Mean values and standard errors from nine experiments with two parallels in each experiment are presented.

The ratio of intra- and extracellular Li^+ concentrations that we observed at Li^+ balanced distribution in U937 cells was near to the value 0.52 obtained for natural Li^+ distribution between human erythrocytes and blood plasma at a very low (0.1–1.0 μM) Li^+ concentration in blood plasma (Fleishman at al., 1984). It is also close to the value of 0.51 observed in patients regularly taking Li^+ and with a concentration thereof in the blood plasma at the level of 0.5–1 mM (Girvin et al., 1996). The coefficient of Li^+/Na^+ discrimination in U937 cells equal to 3 is within the range estimated earlier for this coefficient in the muscle tissue of various marine invertebrates (2–5) and fish (3) (Burovina et al., 1963, 1964; Fleishman et al., 1982). The Li^+ and Na^+ discriminating systems are obviously very conservative.

Kinetics of Li^+ influx and efflux in U937 cells cultured with inhibitors of the Na, K-ATPase pump and Na^+/H^+ exchanger (DMA). Figure 2 shows that blockage of the pump with ouabain does not alter the rate constant of Li^+ efflux. The altered constant for Na^+ efflux registered in the same cells and well-studied drastic decline in the Rb^+ influx in U937 cells produced by ouabain demonstrate that U937 cells are indeed sensitive to ouabain (Vereninov et al., 2007;

Table 1. Li^+/Na^+ discrimination coefficient, rate constants of Li^+ efflux and intracellular K^+, Na^+, and Li^+ content in U937 cells cultivated for a long time in standard RPMI medium and RPMI medium with 10 μM ouabain and 1–10 mM LiCl

Additions in RPMI medium	Li_o^+, mM	Incubation period, h	$\frac{Li_i^+ \cdot Na_o^{+a}}{Na_i^+ \cdot Li_o^+}$	$\frac{Li_i^+}{Li_o^+}$	$k_{Li} \pm$ se min^{-1} $(n)^b$	K^+	Na^+	Li^+
						μmole/g[c]		
LiCl	3	24	3.06 ± 0.11 (8)	0.80 ± 0.05 (8)	0.040 ± 0.005 (9)	687 ± 37 (8)	191 ± 8 (8)	14.0 ± 0.6 (8)
	5	24	2.98 ± 0.08 (58)	0.92 ± 0.03 (43)	0.035 ± 0.002 (20)	892 ± 27 (27)	228 ± 6 (25)	26.6 ± 1 (27)
	5	1.5	2.46 ± 0.13 (34)	0.94 ± 0.03 (37)	0.051 ± 0.003 (28)	808 ± 25 (20)	283 ± 12 (22)	24.7 ± 0.8 (34)
LiCl	1	4	2.96 ± 0.06 (6)	0.71 ± 0.04 (6)	–	970 ± 74 (6)	151 ± 9 (6)	3.6 ± 0.2 (6)
	2	4	3.27 ± 0.09 (5)	0.81 ± 0.05 (5)	–	926 ± 80 (5)	147 ± 13 (5)	7.6 ± 0.5 (5)
	5	4	3.29 ± 0.16 (3)	0.71 ± 0.02 (3)	–	778 ± 16 (3)	124 ± 3 (3)	16.3 ± 0.4 (3)
	10	4	3.64 ± 0.15 (5)	0.84 ± 0.07 (6)	–	879 ± 55 (5)	140 ± 18 (5)	41.6 ± 3.5 (5)
LiCl	5	12	3.32 ± 0.15 (9)	0.95 ± 0.04 (8)	0.042 ± 0.002 (3)	690 ± 13 (6)	168 ± 11 (9)	25.6 ± 0.8 (6)
LiCl + ouabain 4 h	5	12	1.18 ± 0.02 (10)	1.54 ± 0.04 (11)	0.019 ± 0.001 (3)	84.9 ± 1.9 (6)	728 ± 22 (6)	42.0 ± 0.6 (6)
LiCl + ouabain 12 h	5	12	1.08 ± 0.04 (8)	1.54 ± 0.02 (9)	0.015 ± 0.001 (3)	71.3 ± 2.5 (6)	779 ± 51 (6)	42.3 ± 1.6 (6)

[a] The discrimination coefficient was calculated by Li^+ and Na^+ content in the same cells incubated for the indicated time in RPMI medium with Li^+ at indicated concentrations. Li^+ concentration in the medium (Li_o^+) was measured. Li^+ concentration in cells (Li_i^+) was calculated using Li^+ intracellular content and cell water amount estimated by cell buoyant density in Percoll gradient.

[b] To determine the rate constant of Li^+ efflux, the cells were preincubated in the medium with 5 mM LiCl for 12 or 20 h and then transferred into lithium-free medium for 10 or 30 min. k_{Li} was calculated according to the formula $k = -(1/t)\ln y_t/y_0$, where y_t is Li^+ content at time $t = 10$ or 30 min and y_0 is the initial Li^+ content. In ouabain -containing medium, k_{Li} were obtained by approximation of Li^+ efflux curves by the equation $y_t = y_0\exp(-kt)$.

[c] Intracellular ion content was expressed in μmole per 1 g protein. Here and in Tables 2 and 3, the results are presented as mean values and standard errors; (n) number of determinations.

Yurinskaya et al., 2011). It can be concluded that Li^+ is not transported from the cells into the medium with the Na, K-ATPase pump. The same has been observed in many other objects. A small decline in the Li^+ influx was registered in the presence of ouabain in studying of Li^+ gain (Table 2). This shows that the Na, K-ATPase pump is involved in Li^+ transport from the medium into the cells. It is known that, occasionally, Li^+ is able to enter the cells via the potassium pathway of the pump (Dunham and Senyk, 1977; Pandey et al., 1978; Sarkadi et al., 1978). No Li^+/Na^+ discrimination observed in U937 cells after long-term cultivation with ouabain indicates that, under normal conditions, Na^+, but not Li^+, is pumped by the Na, K-ATPase pump. Evidently, Li^+/Na^+ discrimination in other pathways responsible for balanced Li^+ distribution is insignificant.

The Na^+/H^+ exchanger (NHE) plays an important role in Li^+ transport across the cell membrane. Most of its isoforms are blocked with amiloride and its derivatives. Nevertheless, it has been shown that Li^+ transport is not totally blocked with these agents (Grinstein et al., 1984a, 1984b). Dimethylamiloride (DMA) reduced in U937 cells both Na^+ and Li^+ efflux rate (Fig. 2). Relative change was more evident for Li^+ than for Na^+ flux, probably due to the presence in the Na^+ efflux of a component related to the Na,K-ATPase pump not blocked with DMA. A similar decline in Li^+ transfer in the presence of DMA was

Table 2. Li^+ transport rate constant (k_{Li}) estimated by the rate of Li^+ gain with and without inhibitors of the Na/K pump (0.1 mM ouabain) and Na^+/H^+ exchanger (0.05 mM DMA).

Medium	k_{Li}, min^{-1}	Transport inhibited	
		k_{Li}, min^{-1}	k_{Li}, %
RPMI	0.043 ± 0.003 (16)		
RPMI + ouabain	0.036 ± 0.003 (16)	0.007 ± 0.003 (16)	16
RPMI	0.041 ± 0.004 (14)		
RPMI + DMA	0.026 ± 0.001 (14)	0.015 ± 0.004 (14)	37

k_{Li} values were calculated by the formula $y(t) = y_{t=\infty}(1 - \exp(-kt))$, where $y(t)$ is ion content at moment $t = 10$ and $y_{t=\infty}$ is Li^+ content under the balanced state.

Table 3. Metolazone and phloretin have no effect on the rate constant of Li^+ and Cl^- efflux from U937 cells, while Na^+ and Cl^- omission from the medium (MgGluc) changes the rate of Li^+ and Cl^- efflux in opposite directions

Medium	k_{Li}, min^{-1}		k_{Cl}, min^{-1}	
	RPMI	MgGluc	RPMI	MgGluc
RPMI (control)	0.059 ± 0.005	0.019 ± 0.002	0.072 ± 0.009	0.209 ± 0.011
RPMI + metolazone	0.058 ± 0.007	0.023 ± 0.003	0.072 ± 0.005	0.205 ± 0.017
RPMI (control)	0.063 ± 0.014	–	–	–
RPMI + phloretin	0.054 ± 0.007	–	–	–

Cells were preincubated in RPMI medium with addition of 5 mM LiCl for 20 h and $^{36}Cl^-$ for 1.5 h and then transferred into RPMI or 96 mM MgGluc media free of $^{36}Cl^-$ and Li^+ for 10 min. Metolazone and phloretin in concentrations of 30 μM were added into the medium for 10 min. k_{Li} and k_{Cl} values were calculated using the formula $k = -(1/t)\ln y_t/y_0$, where y_t is $^{36}Cl^-$ or Li^+ content at moment $t = 10$ min; y_0 is initial $^{36}Cl^-$ or Li^+ content in cells. The results are mean values with standard errors from two experiments with three parallel measurements in each experiment.

observed in experiments on its entry into the cells (Table 2).

To verify whether amiloride-sensitive Li^+ influx in U937 cells is associated with thiazide-sensitive Na-Cl cotransporter, as has been found in human lens epithelial cells (Lauf et al., 2008), we assayed the impact of metolazone, a specific inhibitor of these transporters considered part of the SLC12A10 family (Gamba, 2009), on the Li^+ transfer. The results presented in Table 3 show that metolazone does not change either Li^+ or Cl^- fluxes. Unlike human erythrocytes and fibroblasts with Li^+ transport being inhibited by phloretin (Pandey et al., 1978; Sarkadi et al., 1978; Duhm and Becker, 1979; Zerbini et al., 1997), the latter agent does not affect Li^+ efflux (Table 3) and influx (data are not presented) in U937 cells.

Influence of Na^+ concentration in the medium on the rate of Li^+ and Na^+ ($^{22}Na^+$) efflux. The relationship between Li^+ and Na^+ transport was shown mostly in experiments on the cross dependence of these cations' transport on their concentration in cells and culture medium. We used the same approach to study Li^+ transport in U937 cells. Figure 3a shows that a decrease in Na^+ concentration to 1 mM and its substitution by Mg^{2+} or $NMDG^+$ is accompanied by a drop in the Na^+ ($^{22}Na^+$) efflux rate constant on average by 40% due to a flux component not inhibited by ouabain. Li^+ efflux rate constant measured under the same conditions also was lower (about 30%) in low-sodium medium than in RPMI medium. Thus, there is a component dependent on Na^+ concentration in the medium.

Figure 3 shows that an increase in Li^+ concentration from 0–5 up 30 mM in Mg-medium results in an increase in the Na^+ efflux constant by 35% (Fig. 3b). This occurs due to an ouabain-resistant component, which predominates when cells are in standard sodium RPMI medium. Thus, under used conditions external Li^+ had an effect on the Na^+ efflux rate, whereas external Na^+ affected the Li^+ efflux rate (Fig. 3).

Measurements of the rate constant of Li^+ and $^{36}Cl^-$ efflux in the same cells showed that the decline in the rate constant of Li^+ efflux in sodium-deficient medium may be produced both by specific flux coupling in the cation exchanger and altered membrane potential. The latter assumption arises if one focuses on the simultaneous threefold opposite change in the rate constant of cation Li^+ and anion Cl^- efflux in cells

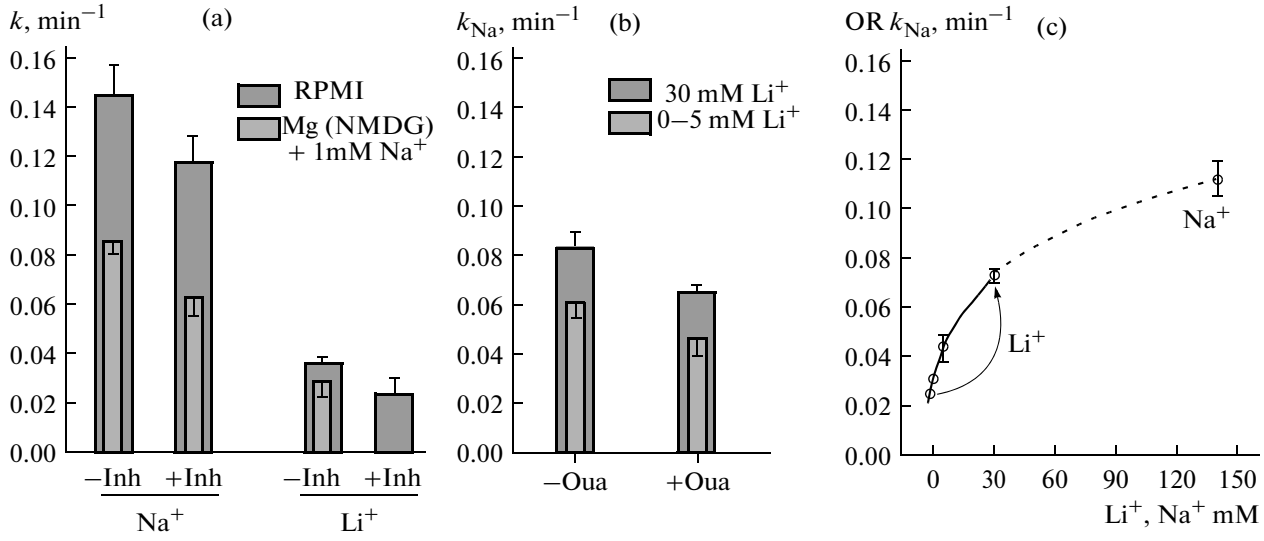

Fig. 3. Rate constants of $^{22}Na^+$ (k_{Na}) and Li^+ (k_{Li}) in U937 cells in RPMI and low-sodium media.
(a) Effect of inhibitors on $^{22}Na^+$ and Li^+ efflux constants in RPMI medium and in media with 1mM Na^+ (96 mM $MgCl_2$ or 140 mM NMDG-Cl + 5.5. mM RbCl + 1mM NaCl). Inhibitors (Inh), mM: 0.1 ouabain + 0.05 bumetanide + 0.05 DMA. The constants were calculated according to the formula $y(t) = y_0\exp(-kt)$, where $y(t)$ is $^{22}Na^+$ or Li^+ content at time $t = 10$ min and y_0 is initial content of the corresponding ion; (b) effect of Li^+ concentration in the Mg-medium on Na^+ rate constant in presence or absence of 0.1 mM ouabain; (c) ouabain-resistant (OR) component of Na^+ constant; first four points (within the range 0–30 mM), Na^+ release in Mg medium with 5.5 mM RbCl + (0–30 mM) LiCl; the last point (140 mM) corresponds to lithium-free sodium medium (RPMI, 140 mM Na^+, 0 Li^+); the constant was determined by $^{22}Na^+$ release. Mean values and standard errors from three experiments with two parallels in each experiment are presented.

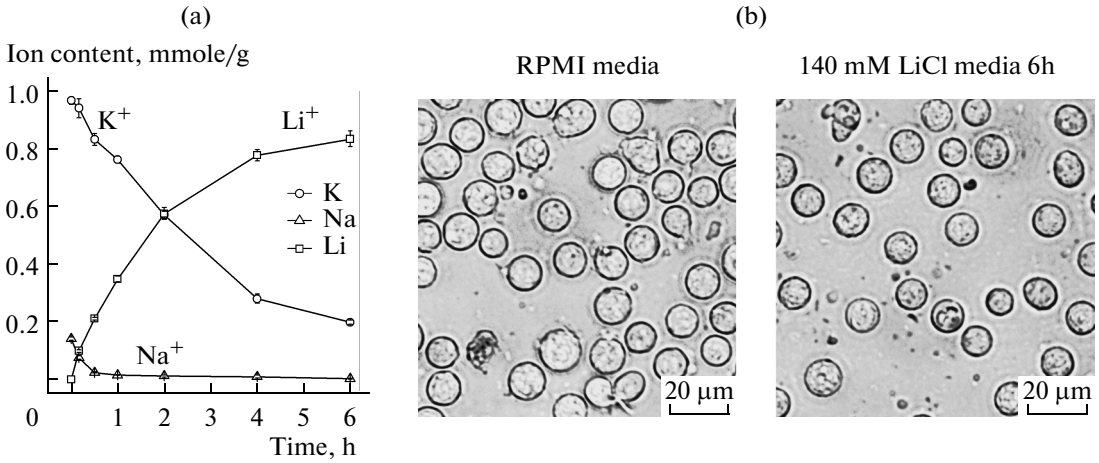

Fig. 4. Content of cations (a) and cell morphology (b) of U937 cell cultures maintained in the medium with Na^+ substituted for Li^+. (a) At 0 time, cells were placed in the medium of the following composition (mM): 140 LiCl, 5.5 KCl, 0.42 $CaCl_2$, 0.41 $MgCl_2$, 10 Hepes + LiOH. Mean values and standard errors from two experiments with two parallels in each experiment are presented; (b) light microscopy of cells cultured in RPMI medium and in Li^+-medium.

transferred into sodium- and chloride-free MgGluc medium (Table 3). This indicates that it is necessary to analyze very carefully the data obtained after Na^+ substitution by Mg^{2+} in the culture medium. These data are frequently used for the validation of Li^+/Na^+ countertransport activity (see, for example: Schork et al., 2002).

Na^+ and K^+ substitution by lithium during long-term incubation of U937 cells in medium with high Li^+ concentration. What is the monovalent ion distribution between cells and medium if Na^+ capable of being pumped from the cells has been substituted by Li^+ unable to be pumped either with the Na, K-ATPase pump or the coupled Na^+/Li^+ exchange system because of a lack of Na^+ in the medium? Gradual sub-

stitution of intracellular Na^+ and K^+ for Li^+ appeared to occur under these conditions in U937 cells (Fig. 4a). For 5–6 h, almost equivalent substitution of these cations is observed. At the same time, the intracellular water content remains on the initial level. In this case, it was 5.16 ± 0.06 mL/g in RPMI medium and 5.15 ± 0.10 mL/g in Li medium ($n = 8$).

The question arises of how these cells endure almost total substitution of intracellular Na^+ and K^+ for Li^+. Routine light microscopy of U937 cells cultivated in the lithium medium for 5–6 h did not show remarkable alteration of cell morphology (Fig. 4b). The number of damaged cells (stained with EB but not with AO) increased insignificantly, from 5.1 ± 0.7 to $11.5 \pm 2.1\%$ (dead cell mean values and their standard errors in three independent experiments). These "lithium" cells are of great interest as model objects to study the role of K^+ and Na^+ in the regulation of various cellular processes and cell volume in hypo- and hyperosmotic media, in particular.

Determination of the rate of Na^+/Li^+ substitution in cells after the total external Na^+/Li^+ substitution is used commonly as a method to evaluate the activity of Li^+/Na^+ countertransport. According to our findings, Na^+ efflux accompanied by simultaneous increase in Li^+ content is only the initial phase of intracellular cation substitution for Li^+. The mathematical model of ion-balance rearrangement after turning off the sodium pump shows that a reciprocal increase in the intracellular Li^+ concentration and decrease in Na^+ and K^+ concentration is observed in the absence of the Li^+/Na^+ countertransport system (Armstrong, 2003, our own findings).

DISCUSSION

An extensive literature is devoted to Li^+ transport, especially in human blood and epithelial cells. It is known that Li^+/Na^+ countertransport is modified by hypertension (Canessa et al., 1980; West et al., 1998; Semplicini et al., 2003; Zheng et al., 2009), diabetes, nephropathy (Zerbini et al., 2003, 2004), and other pathologies. Li^+ therapy is used in psychiatry (Lenox, Le Wang, 2003; El Balkhi et al., 2009). Key questions in our work are what the resemblances and differences are in Li^+ and Na^+ transport across the cell membrane in U937 cells and what the balance is of Li^+ and Na^+ influx and efflux in view of the data obtained.

Four types of Li^+ transfer across the cell membrane have been determined since the 1980s. They are Li^+ countertransport (about 75% of efflux in erythrocytes) (Ehrlich and Diamond, 1979), Li^+ transfer via a bicarbonate-dependent system (about 30% of efflux), and "leakage" through channels and under certain conditions through the Na, K-pump (see also: Pandey et al., 1978; Sarkadi et al., 1978; Duhm and Becker, 1979). A similar scheme was attributed to cultured neuroblastoma cells (Reiser and Duhm, 1982). Later, this was confirmed for other cells and additional Li^+/Na^+ countertransport not inhibited by ouabain and amiloride was reported for erythrocytes (Canessa, 1989; West et al., 1998). Currently, it is still unclear what transporter associated with Li^+/Na^+ countertransport is engaged in hypertension (Timmer and Sands, 1999; Elmariah and Gunn, 2003; Xiang et al., 2007). Genetic studies did not identify the gene responsible for modified Li^+/Na^+ countertransport under hypertension (Lifton et al., 1991; Lifton, 1996; Schork et al., 2002).

In human erythrocytes and other cells, NHE plays a major role in Li^+/Na^+ countertransport (Kemp et al., 2008). However, NHE is not the only transporter in which Na^+ is able to substitute Na^+ for Li^+. Coupled Li^+/Na^+ flux is possible in symporter NKCC (Lytle et al., 1998) and other transporters, Na(Li)-phosphate symporter in particular (Timmer and Gunn, 2000; Elmariah and Gunn, 2003; Andrini et al., 2012). It is possible that, in cells of various species and tissue origin, different transporters may be involved in lithium transfer across the plasma membrane. For example, Li^+ may substitute for Na^+ in bicarbonate-dependent Na^+ transport in erythrocytes but not in U937 cells (Ladoux et al., 1987). A more complicated situation has been reported: in canine erythrocytes, Na^+ activates NHE exchanger and is transported by this system through the sites involved in blocking the exchanger with lithium (Dunham et al., 2005).

The data we obtained show that, in U937 cells, Na^+, but not Li^+, is pumped out of the cytoplasm by the Na, K-ATPase pump. These cells are quite similar to other cells having a Na, K-ATPase pump. It is known that, under certain "not physiological" conditions, the Na, K-ATPase pump may be activated by lithium, which makes possible lithium transport via the pump out of the cell but more frequently into the cell (Dunham and Senyk, 1977; Sarkadi et al., 1978; Ehrlich and Diamond, 1979). We also observed ouabain-inhibited Li^+ influx in U937 cells. Although the inhibitory effect of DMA indicates both Li^+ and Na^+ to be transported by Na^+/H^+ exchanger in U937 cells, they may also be transported by the amiloride-insensitive isoform of the Na^+/H^+ exchanger. The involvement of this isoform in $Li^+$and Na^+ transport has been shown in human and hamster fibroblasts (Zerbini et al., 1997; 2001; 2003, 2004) and other cells (Orlowski and Grinstein, 2004; Brett et al., 2005; Xiang et al., 2007; Kemp et al., 2008; Schushan et al., 2010). As it will be shown below, Li^+ influx is too high to be related to electrodiffusion channels.

Phloretin, a inhibitor-marker of Li^+/Na^+ countertransport in erythrocytes (Sarkadi et al., 1978; Duhm, Becker, 1979), and metolazone, a specific blocker of thiazide-sensitive Na-Cl cotransport (Lauf et al., 2008; Gamba, 2009) did not affect Li^+ and Na^+ fluxes

Table 4. The balance of unidirectional Na^+ and Li^+ fluxes in U937 cells equilibrated with the medium at 5 mM Li^+ and 140 mM Na^+

Ions	Fluxes, μmole g^{-1} min^{-1} (%)				Symporter[b]
	tracts	efflux	influx	net	
Na^+	All[a]	42 (100%)	42	0	
	Pump	5.5 (13%)	0	5.5	
	Channels	0.16–0.4	2–5	1.84–4.6	NC = 4–1, NKCC = 0
	NC	0.4–0.1	4–1	3.6–0.9	
	Channels	0.6	6 (14%)	5.4	NC = 0, NKCC = 0.15
	NKCC	0.11	0.15	0.04	
	Na^+/Na^+	≈36 (87%)	≈36	0	
Li^+	All	1.12 (100%)	1.12	≈0	
	Channels	0.007–0.018	0.072–0.18	0.07–0.16	NC = 4–1, NKCC = 0
	NC	0.014–0.004	0.14–0.036	0.13–0.032	
	Channels	0.022	0.22	0.19	NC = 0, NKCC = 0.15
	NKCC	0.004	0.0054	0.0014	
	Li^+/Li^+	0.90 (80%)	0.90	0	
	Li^+/Na^+	≈0.2	0	≈0.2	

[a] Experimental values of the total Na^+ and Li^+ fluxes were obtained by multiplication of the rate constant of ion exchange on intracellular ion content. Unidirectional Na^+ fluxes were calculated as described in (Yurinskaya et al., 2011). Na^+ efflux via pump was estimated by the ouabain-inhibited Rb^+ influx. Unidirectional Li^+ influxes through channels and symporters were estimated as Na^+ influxes but with another external concentration, 5 mM for Li^+ instead of 140 mM for Na^+. Li^+ efflux was evaluated on the assumption that the influx/efflux ratio for Na^+ and Li^+ in all ion pathways except the pump is similar.

[b] Symport type. Two values of NC symport correspond to various flux values.

in U937 cells. In some cells Li^+ like Na^+ may be transported by NKCC. Bumetanide is a specific inhibitor of NKCC transporter. The detailed study was carried out on duck erythrocytes (Lytle et al., 1998). We have carried out many experiments on bumetanide influence on monovalent ion transport in U937 cells. It was found that, under certain conditions, bumetanide reduced only Rb^+ influx, applied as a marker of K^+ flux (Vereninov et al., 2008). No changes in Li^+ flux were registered in U937 cells under the same conditions. Thus, it is very difficult to determine the transporter for coupling Li^+ flux counter electrochemical gradient with Na^+ flux in U937 cells using the inhibitor analysis.

How may analysis of Li^+ balanced distribution be useful? Li^+ exchange between the cytoplasm and medium occurs one or two orders more rapidly in proliferating cells than in erythrocytes. In this case, examinations of ion fluxes may be carried out under conditions of balanced ion distribution. This facilitates the analysis of mechanisms of ion transport across the cell membrane. However, Li^+ transport in proliferating cells in culture has been poorly studied. Early studies on Li^+ transport in HeLa cells (Boardman et al., 1975), neuroblastoma (Reise and Duhm, 1982), and L6 myoblasts (Laurenz and Smith, 1998) and later studies of Na(Li)-phosphate cotransporter in K562 cells (Timmer and Gunn, 2000), Li^+ transport in human fibroblasts (Zerbini et al., 1997; 2001) and coupling Li^+ and Na^+ flux with Na^+ and Cl^- cotransporter in cultured human lens epithelium (Lauf, 2008) should be mentioned. Flux balance of all three monovalent ions Na^+, K^+, and Cl^- is the most comprehensively studied in U937 cells (Yurinskaya et al., 2005; Vereninov et al., 2007; Yurinskaya et al., 2011).

The general Na^+ influx and efflux determined by radiotracer and flame-emission analysis is determined in U937 cells under normal conditions as 42 μmole g^{-1} min^{-1} (Vereninov et al., 2007). The calculations of unidirectional Na^+, K^+, and Cl^- fluxes (Yurinskaya et al., 2011) via the main tracts, i.e., through the pump, channels, and NC and NKCC symporters, under conditions when all three ion fluxes are balanced show that Na^+ efflux through the pump is about 13% in U937 cells (Table 4). Most of the Na^+ influx down the electrochemical gradient is mediated by channels; the rest falls on symporters NC and NKCC. Together, these fluxes are balanced by Na^+ efflux via the pump. The other 87% of the general flux is Na^+/Na^+ equivalent exchange that is intrinsically balanced.

It can be stated that, in channels and symporters, Li^+ behaves as a close analogue of Na^+ (Lytle et al., 1998; Hille, 2001). In this case, the Li^+ net flux down the gradient should differ from Na^+ flux as their external concentrations differ (5 : 140 = 0.036). The difference in the intracellular Li^+ and Na^+ concentrations may be neglected, because the backward fluxes of these ions are approximately ten times less than the forward ones and the intracellular concentrations practically do not influence the net fluxes (Yurynskaya et al., 2011). According to these estimations, the total Li^+ net influx down the electrochemical gradient is no more than 0.2 μmole g^{-1} min^{-1}. This flux may be compensated by an Li^+ efflux counter gradient coupled with Na^+ influx.

In conclusion, in U937 cells, under conditions close to physiological ones with 5 mM Li^+ in the medium and its balanced distribution between the cells and medium (Li^+ influx and efflux via the all tracts is 1.1, Na^+ flux is 42 μmole g^{-1} min^{-1}), about 80% of the Li^+ flux falls on the equivalent Li^+/Li^+ transport. The portion of Li^+ net influx down the gradient is about 20%. The majority accounts for channels, and less is associated with influx via symporters NC or NKCC. This influx is compensated by Li^+ efflux counter gradient of electrochemical potential involved in Li^+/Na^+ exchange comprised 0.5% of the total Na^+ flux.

Coupled Na^+/Na^+ or Li^+/Li^+ counterfluxes cannot be revealed with electrophysiological methods or by determination of total Na^+ or total Li^+ content in cells. The study of Na^+ and Li^+ transport shows that there are no blockers able to inhibit $Na^+/Na^+/Li^+$ exchange. Obviously, Li^+ is a valuable ion marker and suitable instrument to study Na^+/Na^+ exchange and Na^+/Li^+ exchange. The other conclusion we draw from our study is that the cells incubated for a long time in the medium with Na^+ substituted by Li^+ sustain the water balance and do not exhibit damages or apoptosis identified by AO and EB. These "lithium" cells may be used as a a model object to study the regulation of monovalent ion transport under a blocked Na, K-ATPase pump and extremely low K^+ and Na^+ intracellular concentration.

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REFERENCES

Andrini, O., Meinild, A.K., Ghezzi, C., Murer, H., and Forster, I.C., Lithium interactions with Na^+-coupled inorganic phosphate cotransporters: insights into the mechanism of sequential cation binding, *Am. J. Physiol. Cell. Physiol.*, 2012, vol. 302, no. 3, pp. C539–C554.

Armstrong, C.M., The Na,K pump, Cl ion, and osmotic stabilization of cells, *Proc. Natl. Acad. Sci. USA.*, 2003, vol. 100, pp. 6257–6262.

Boardman, L.J., Hume, S.P., Lamb, J.F., and Polson, J., Effect of growth in lithium on ouabain binding, Na-K-ATPase and Na and K transport in HeLa cells, *J. Physiol.*, 1975, vol. 244, pp. 677–682.

Brett, C.L., Donowitz, M., and Rao, R., Evolutionary origins of eukaryotic sodium/proton exchangers, *Am. J. Physiol. Cell Physiol.*, 2005, vol. 288, pp. C223–C239.

Burovina, I.V., Glazunov, V.V., Leont'ev, V.G., Nesterov, V.P., and Skul'skii, I.A., Alkali elements in the evolution of marine organisms, *Zh. Obshch. Biol.*, 1964, vol. 25, no. 2, pp. 115–122.

Burovina, I.V., Glazunov, V.V., Leont'ev, V.G., Nesterov, V.P., Skul'skii, I.A., Fleishman, D.G., and Shmitko, M.N., Content of lithium, sodium, potassium, rubidium, and cesium in the muscles of marine organisms in the Barents and Black Seas, *Dokl. Akad. Nauk SSSR*, 1963, vol. 149, no. 2, pp. 413–415.

Canessa, M., Adragna, N., Solomon, H.S., Connolly, T.M., and Tosteson, D.C., Increased sodium-lithium countertransport in red cells of patients with essential hypertension, *N. Engl. J. Med.*, 1980, vol. 302, pp. 772–776.

Canessa, M., Kinetic properties of Na^+/H^+ and Li^+/Na^+, Na^+/Na^+ and Na^+/Li^+ exchanges of human red cells, *Methods Enzymol.*, 1989, vol. 173, pp. 176–191.

Duhm, J. and Becker, B.F., Studies on lithium transport across the red cell membrane. V. On the nature of the Na^+-dependent Li^+ countertransport system of mammalian erythrocytes, *J. Membr. Biol.,* 1979, vol. 51, pp. 263–286.

Dunham, P.B., and Senyk, O., Lithium efflux through the Na/K pump in human erythrocytes, *Proc. Natl. Acad. Sci. USA.*, 1977, vol. 74, pp. 3099–3103.

Dunham, P.B., Scott, J.K., Logue, P.J., Mutolo, M.J., and Milanick, M.A., Na^+-inhibitory Sites of the Na^+/H^+ Exchanger are Li^+ Substrate Sites, *Am. J. Physiol. Cell Physiol.*, 2005, vol. 289, pp. C277–C282.

Ehrlich, B.E. and Diamond, J.M., Lithium fluxes in human erythrocytes, *Am. J. Physiol. Cell. Physiol.*, 1979, vol. 237, pp. C102–C110.

El Balkhi, S., Megarbane, B., Poupon, J., Baud, F.J., and Galliot-Guilley, M., Lithium poisoning: is determination of the red blood cell lithium concentration useful? *Clin. Toxicol.*, 2009, vol. 47, pp. 8–13.

Elmariah, S.and Gunn, R.B., Kinetic evidence that the $Na-PO_4$ cotransporter is the molecular mechanism for Na/Li exchange in human red blood cells, *Am. J. Physiol. Cell Physiol.*, 2003, vol. 285, pp. C446–C456.

Fleishman, D.G., *Schelochnye elementy i ikh radioaktivnye izotopy v vodnykh ekosistemakh* (Alkaline Elements and Their Radioactive Isotopes in Aquatic Ecosystems), Leningrad: Nauka, 1982.

Fleishman, D.G., Arutyunyan, V.M., Bagrov, Ya.Yu., Gurevich, Z.P., Drampyan, F.S., and Solyus, A.A., Lithium content in the blood of people from different regions of the USSR, *Dokl. Akad. Nauk SSSR,* 1984, vol. 279, no. 3, pp. 758–761.

Gamba, G., The thiazide-sensitive Na-Cl cotransporter: molecular biology, functional properties, and regulation by

WNKs, *Am. J. Physiol. Renal. Physiol.*, 2009, vol. 297, pp. F838–F848.

Girvin, B., Byrne, A., King, D.J, Wilson-Davis, K., and Johnston, G.D., An assessment of erythrocyte lithium concentrations as a measure of patient compliance, *Hum. Psychopharmacol.*, 1996, vol. 11, pp. 491–495.

Grinstein, S., Goetz, J.D., and Rothstein, A., $^{22}Na^+$ Fluxes in thymic lymphocytes. I. Na^+/Na^+ and Na^+/H^+ exchange through an amiloride-insensitive pathway, *J. Gen. Physiol.*, 1984a, vol. 84, pp. 565–584.

Grinstein, S., Goetz, J.D., and Rothstein, A., $^{22}Na^+$ Fluxes in thymic lymphocytes. II. Amiloride-sensitive Na^+/H^+ exchange pathway; reversibility of transport and asymmetry of the modifier site, *J. Gen. Physiol.*, 1984b, vol. 84, pp. 585–600.

Hille, B., *Ion Channels of Excitable Membranes*, 3rd ed., Sunderland, MA: Sinauer Associates, Inc., 2001.

Kemp, G., Young, H., and Fliegel, L., Structure and function of the human Na^+/H^+ exchanger isoform 1, *Channels*, 2008, vol. 2, pp. 329–336.

Ladoux, A., Krawice, I., Cragoe, E.J., Jr., Abita, J.P., and Frelin, C., Properties of the Na+-dependent Cl^-/HCO_3^- exchange system in U937 human leukemic cells, *Eur. J. Biochem.*, 1987, vol. 170, pp. 43–49.

Lauf, P.K., Chimote, A.A., and Adragna, N.C., Lithium fluxes indicate presence of Na-Cl cotransport (NCC) in human lens epithelial cells, *Cell. Physiol. Biochem.*, 2008, vol. 21, pp. 335–346.

Laurenz, J.C. and Smith, S.B., Lithium chloride does not inhibit the proliferation of L6 myoblasts by decreasing intracellular free inositol, *J. Anim. Sci.*, 1998, vol. 76, pp. 66–73.

Lenox, R.H. and Le Wang, Molecular basis of lithium action: integration of lithium-responsive signaling and gene expression networks, *Mol. Psychiatry*, 2003, vol. 8, pp. 135–144.

Lifton, R.P., Hunt, S.C., Williams, R.R., Pouysségur, J., and Lalouel, J.M., Exclusion of the Na^+–H^+ antiporter as a candidate gene in human essential hypertension, *Hypertension*, 1991, vol. 17, pp. 8–14.

Lifton, R.P., Molecular genetics of human blood pressure variation, *Science*, 1996, vol. 272, pp. 676–680.

Lytle, C., McManus, T.J., and Haas, M., A model of Na-K-2Cl cotransport based on ordered ion binding and glide symmetry, *Am. J. Physiol. Cell Physiol.*, 1998, vol. 274, pp. C299–C309.

Matthopoulos, D.P., Tzaphlidou, M., and Leontiou, I., Morphological alterations caused by lithium in various cell lines, *Cell Biol. Int.*, 1995, vol. 19, pp. 499–506.

Mills, B. and Tupper, J.T., Cation permeability and ouabain-insensitive cation flux in the Ehrlich ascites tumor cell, *J. Membr. Biol.*, 1975, vol. 20, pp. 75–97.

Orlowski, J. and Grinstein, S., Diversity of the mammalian sodium/proton exchanger SLC9 gene family, *Pflugers Arch.*, 2004, vol. 447, pp. 549–565.

Pandey, G.N., Sarkadi, B., Haas, M., Gunn, R.B., Davis, J.M., and Tosteson, D.C., Lithium transport pathways in human red blood cells, *J. Gen. Physiol.*, 1978, vol. 72, pp. 233–247.

Reiser, G. and Duhm, J., Transport pathways for lithium ions in neuroblastoma x glioma hybrid cells at "therapeutic"' concentrations of Li^+, *Brain Research*, 1982, vol. 252, pp. 247–258.

Sarkadi, B., Alifimoff, J.K., Gunn, R.B., and Tosteson, D.C., Kinetics and stoichiometry of Na-dependent Li transport in human red blood cells, *J. Gen. Physiol.*, 1978, vol. 72, pp. 249–265.

Schork, N.J., Gardner, J.P., Zhang, L., Fallin, D., Thiel, B., Jakubowski, H., and Aviv, A., Genomic/association linkage of sodium lithium countertransport in CEPH pedigrees, *Hypertension*, 2002, vol. 40, pp. 619–628.

Schushan, M., Xiang, M., Bogomiakov, P., Padan, E, Rao, R., and Ben-Tal, N., Model-guided mutagenesis drives functional studies of human NHA2, implicated in hypertension, *J. Mol. Biol.*, 2010, vol. 396, pp. 1181–1196.

Semplicini, A., Sartori, M., Ceolotto, G., and Caló, L.A., The Li^+/Na^+ Exchange in Hypertension, *Front Biosci.*, 2003, vol. 8, pp. d912–d929.

Timmer, R.T. and Gunn, R.B., The molecular basis for na-dependent phosphate transport in human erythrocytes and K562 cells, *J. Gen. Physiol.*, 2000, vol. 116, pp. 363–378.

Timmer, R.T. and Sands, J.M., Lithium intoxication, *J. Am. Soc. Nephrol.*, 1999, vol. 10, pp. 666–674.

Tupper, J.T., Cation flux in the Ehrlich ascites tumor cell. Evidence for Na^+-for-Na^+ and K^+-for-K^+ exchange diffusion, *Biochim. Biophys. Acta*, 1975, vol. 394, pp. 586–596.

Vereninov, A.A., Goryachaya, T.S., Matveev, V.V., Moshkov, A.V., Rozanov, Yu.M., Sakuta, G.A., Shirokova, A.V., and Yurinskaya, V.E., Cell shrinkage during apoptosis is not obligatory. Apoptosis of U937 cells induced by staurosporine and etoposide, *Tsitologiia*, 2004, vol. 46, no. 7, pp. 609–619.

Vereninov, A.A., Goryachaya, T.S., Moshkov, A.V., Vassilieva, I.O., Yurinskaya, V.E., Lang, F., and Rubashkin, A.A., Analysis of the monovalent ion fluxes in U937 cells under the balanced ion distribution: recognition of ion transporters responsible for changes in cell ion and water balance during apoptosis, *Cell Biol. Int.*, 2007, vol. 31, pp. 382–393.

Vereninov, A.A., Rubashkin, A.A., Goryachaya, T.S., Moshkov, A.V., Rozanov, Y.M., Shirokova, A.V., Strelkova, E.G., Lang, F., and Yurinskaya, V.E., Pump and channel $K(Rb^+)$ fluxes in apoptosis of human lymphoid cell line U937, *Cell. Physiol. Biochem.,* 2008, vol. 22, pp. 187–194.

West, I.C., Rutherford, P.A., and Thomas, T.H., Sodium-lithium countertransport: physiology and function, *J. Hypertens.*, 1998, vol. 16, pp. 3–13.

Xiang, M., Feng, M., Muend, S., and Rao, R., A human Na^+/H^+ antiporter sharing evolutionary origins with bacterial NhaA may be a candidate gene for essential hypertension, *Proc. Natl. Acad. Sci. USA*, 2007, vol. 104, pp. 18677–18681.

Yurinskaya, V., Goryachaya, T., Guzhova, I., Moshkov, A., Rozanov, Y., Sakuta, G., Shirokova, A., Shumilina, E., Vassilieva, I., Lang, F., and Vereninov, A., Potassium and sodium balance in U937 cells during apoptosis with and without cell shrinkage, *Cell. Physiol. Biochem.*, 2005, vol. 16, pp. 155–162.

Yurinskaya, V.E., Goryachaya, T.S., Rubashkin, A.A., Shirokova, A.V., and Vereninov, A.A., Changes in K^+, Na^+, Cl^- balance and K^+, Cl^- fluxes in U937 cells induced to apoptosis by staurosporine: on cell dehydration in apoptosis, *Cell Tissue Biol.*, 2010, vol. 4, no. 5, pp. 457–463.

Yurinskaya, V.E., Rubashkin, A.A., and Vereninov, A.A., Balance of unidirectional monovalent ion fluxes in cells undergoing apoptosis: why does Na^+/K^+ pump suppression not cause cell swelling? *J. Physiol.*, 2011a, vol. 589, pp. 2197–2211.

Yurinskaya, V.E., Rubashkin, A.A., Shirokova, A.V., and Vereninov, A.A., Regulatory volume increase (RVI) and apoptotic volume decrease (AVD) in U937 cells in hypertonic medium, *Cell Tissue Biol.*, 2011b, vol. 5, no. 5, pp. 487–494.

Zerbini, G., Mangili, R., Gabellini, D., and Pozza, G., Modes of operation of an electroneutral Na^+/Li^+ countertransport in human skin fibroblasts, *Am. J. Physiol. Cell Physiol.*, 1997, vol. 272, pp. C1373–C1379.

Zerbini, G., Podesta, F., Meregalli, G., Deferrari, G., and Pontremoli, R., Fibroblast Na-Li countertransport rate is elevated in essential hypertension, *J. Hypertens.*, 2001, vol. 19, pp. 1–7.

Zerbini, G., Maestroni, A., Breviario, D., Mangili, R., and Casari, G., Alternative splicing of NHE-1 mediates Na–Li countertransport and associates with activity rate, *Diabetes*, 2003, vol. 52, pp. 1511–1518.

Zerbini, G., Gabellini, D., Ruggieri, D., and Maestroni, A., Increased sodium-lithium countertransport activity: a cellular dysfunction common to essential hypertension and diabetic nephropathy, *J. Am. Soc. Nephrol.*, 2004, vol. 15, pp. S81–S84.

Zheng, X., Kammerer, C.M., Cox, L.A., Morrison, A., Turner, S.T., and Ferrell, R.E., Association of SLC34A2 variation and sodium-lithium countertransport activity in humans and baboons, *Am. J. Hypertens.*, 2009, vol. 22, pp. 288–293.

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