

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

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Abstract

Unidirectional ^{22}Na , Li^+ and Rb^+ fluxes and net fluxes of Na^+ and K^+ were measured in U937 human leukemic cells before and after induction of apoptosis by staurosporine (1 μM , 4 h) to answer the question which ion transporter(s) are responsible for changes in cell ion and water balance at apoptosis. The original version of the mathematical model of cell ion and water balance was used for analysis of the unidirectional ion fluxes under the balanced distribution of major monovalent ions across the cell membrane. The values of all major components of the Na^+ and K^+ efflux and influx, i.e. fluxes via the Na^+, K^+ -ATPase pump, Na^+ channels, K^+ channels, Na/Na exchanger and $\text{Na}-\text{Cl}$ symport were determined. It is concluded that apoptotic cell shrinkage and changes in Na^+ and K^+ fluxes typical of apoptosis in U937 cells induced by staurosporine are caused by a complex decrease in the pump activity, $\text{Na}-\text{Cl}$ symport and integral Na^+ channel permeability.
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Keywords: Na^+ flux; Rb^+ flux; K^+ flux; Li^+ flux; U937 cells; Apoptosis; Cell ion balance; Cell water balance

1. Introduction

The pump-leak steady-state concept for control of cell ion and water balance is as old as the idea of the ion pump (Dean, 1941, 1987; Krogh, 1946; Leaf, 1959; Hoffmann, 2001; Stein, 2002). Integrated mathematical models of cell volume, pH and ion content regulation have been developed since then, delivering many unexpected predictions (Tosteson and Hoffman, 1960; Jakobsen, 1980; Lew and Bookchin, 1986; Vereninov and Marakhova, 1986; Hernandez and Cristina, 1998). Being based on the same general principles, these models differ in the ways of matching calculated and experimental data. Here we demonstrate the model for analysis of the mechanisms maintaining ion and water balance in human U937 leukemic cells in two different states: before and after induction of apoptosis. The central point of our study was the measurement of unidirectional fluxes of the

monovalent cations: $^{22}\text{Na}^+$, Li^+ as a non-pumped analogue of Na^+ , and Rb^+ used as a congener of K^+ . Therefore we considered changes in the major characteristics of ion and water balance in the cell model as a function of the measurable ion fluxes rather than the kinetic parameters determined by calculation. The values of all major components of the Na^+ and K^+ efflux and influx, i.e. fluxes via the Na^+, K^+ -ATPase pump, Na^+ , K^+ channels, Na/Na exchanger and $\text{Na}-\text{Cl}$ symport were determined by using both the experimental data and mathematical modeling. It is concluded that apoptotic cell shrinkage and changes in Na^+ and K^+ fluxes typical for apoptosis of U937 cell induced by staurosporine are caused by a decrease in the pump activity, $\text{Na}-\text{Cl}$ symport and integral Na^+ channel permeability.

2. Materials and methods

2.1. Reagents

RPMI 1640 medium and fetal bovine serum (FBS) were from Biolot (Russia). Staurosporine (STS), ouabain, 5-(*N,N*-dimethyl)-amiloride (DMA),

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4,4-diisothiocyanatostilbene-2,2-disulfonic acid (DIDS), *R*-(+)-[(2-*n*-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1H-inden-5-yl)oxy]acetic acid (DIOA) were from Sigma–Aldrich (Germany). For the generation of stock solutions of DMA (10 mM) and DIOA (50 mM) were dissolved in DMSO, ouabain (1 mM) in phosphate salt buffer solution (PBS) and DIDS (20 mM) in water. The final concentration of DMSO was 0.2% and 0.5% in the case of DIOA and DMA, respectively. Percoll was from Pharmacia (Sweden). Isotope $^{22}\text{Na}^+$ was from “Isotope” (Russia). Salts were analytical grade from Reachim (Russia).

2.2. Cell culture

The U937 cell line was obtained from the Russian cell culture collection (Institute of Cytology, Russian Academy of Sciences). Some $(0.7–1.0) \times 10^6$ cells ml^{-1} were incubated in RPMI medium at 37 °C and in 5% CO_2 in air. For the induction of apoptosis, the cells were treated with 1 μM STS for 4 h. A stock solution of STS (2 mM) in DMSO was diluted to yield a final concentrations of the drug of 1 μM . The final concentration of DMSO was 0.05%.

2.3. Determination of intracellular ion content

Cells were pelleted in RPMI medium and washed in MgCl_2 solution (96 mM) 5 times without resuspending. The pellets were treated with 1 ml of 5% trichloroacetic acid (TCA) for 30 min and TCA-extracts were analyzed for $[\text{K}^+]$, $[\text{Na}^+]$, $[\text{Li}^+]$ and $[\text{Rb}^+]$ by emission photometry in air-propane flame by using a Perkin–Elmer AA 306 spectrophotometer and solutions of KCl or NaCl (10–100 μM) and LiCl or RbCl (5–10 μM) in 5% TCA as a standard. The TCA precipitates were dissolved in 0.1 N NaOH and analyzed for protein by the Lowry procedure with serum bovine albumin as a standard. The cell ion content was calculated in μmol per g of protein.

2.4. Study of the $^{22}\text{Na}^+$, Li^+ , Rb^+ uptake and release from cells

To study the time course of $^{22}\text{Na}^+$, Li^+ , Rb^+ uptake aliquots of $\approx 1 \times 10^6$ cells (1 ml) were pelleted and resuspended in 0.5 ml of RPMI medium containing ^{22}Na (4.8×10^6 cpm ml^{-1}), 5 mM LiCl and 2.5 mM RbCl (37 °C) for 3–20 min. The cells were then sedimented, washed in 96 mM MgCl_2 solution 5 times without resuspending and used for ion determination.

To study the rate of $^{22}\text{Na}^+$, Li^+ and Rb^+ release 2×10^6 cells ml^{-1} were loaded in RPMI medium with $^{22}\text{Na}^+$ (4.8×10^6 cpm ml^{-1}) and 5 mM LiCl for 1.5 h at 37 °C or $0.7–1 \times 10^6$ cells ml^{-1} cultured under standard conditions 24 h in RPMI medium with 1 mM RbCl and 3–5 mM LiCl. The cells were then sedimented, washed in 96 mM MgCl_2 solution without resuspending 5 times and resuspended in $^{22}\text{Na}^+$, Li^+ , Rb^+ - free RPMI medium to 1×10^6 cells ml^{-1} for 10 min at 37 °C. The cells were sedimented, the supernatant and the cell pellet were counted for $^{22}\text{Na}^+$ determinations and used for Li^+ and Rb^+ measurements. The results were analyzed by Student's *t*-test and considered significantly different at $P < 0.05$.

2.5. Calculation of fluxes and rate constants of ion efflux and influx. General definitions

It is assumed that the uptake and the release of small quantities of the ions used as tracers under the balanced distribution of the major ions follow a simple exponential kinetic, described by the equations: $y(t) = y_1 = \infty (1 - \exp(-k_{io}t))$ for ion gain and $y(t) = y_0 \exp(-k_{io}t)$ for ion exit, where $y(t)$ is the tracer content at the time t , $y_1 = \infty$ and y_0 are the final and initial intracellular tracer contents, and k_{io} is the rate constant of ion equilibration. The rate constant k_{io} in both of these equations is one and the same parameter which corresponds to the “efflux rate constant”, defined as a ratio of the efflux (m_{io}) to the intracellular ion content: $k_{io} = m_{io}/\text{Na}_i^+$. As the errors in the experimental determination of k_{io} by analysis of the time course of the ion uptake and release depend on the chosen time interval and can be different, the values of the constant found by these two methods appeared to be different. The efflux rate constants found by the best approximation of the experimental data

with the equations for the tracer release and uptake were designated as $*k_{io}$ and $**k_{io}$, respectively. The total Na^+ (Li^+ , Rb^+) efflux was calculated as $k_{io} \text{Na}_i^+$ (Li^+ , Rb^+), where Na_i^+ (Li^+ , Rb^+) is the ion content per g of cell protein.

To determine changes in the “influx rate constant” (k_{oi}) at apoptosis, it was considered that under the balanced state the following relationships should hold:

$$k_{oi}/k_{io} = \text{Na}_i^+/\text{Na}_o^+ \quad (1)$$

$$k_{oi}^A/k_{io}^A = \text{Na}_i^{+A}/\text{Na}_o^{+A} \quad (2)$$

where superscript A corresponds to the apoptotic cells. From Eqs. (1) and (2) it follows that under the constant Na_o^+ the changes in Na_i^+ equals the changes in the ratios of the influx and efflux rate constants

$$\text{Na}_i^{+A}/\text{Na}_i^+ = (k_{oi}^A/k_{oi})/(k_{io}^A/k_{io}) \quad (3)$$

The relationship (3) means that the intracellular ion content is not changed when the rate constants k_{oi} and k_{io} vary to the same extent. The relationships similar to Eqs. (1)–(3) hold for other ions: Li^+ , Rb^+ , and K^+ .

The following components of the total fluxes of Na^+ and K^+ (Rb^+) were taken into consideration as the major components:

$$\{\text{Na}^+\text{efflux}\} = \text{ENaP} + \text{ENaG} + \text{ENaS} + \text{ENaNa} \quad (4a)$$

$$\{\text{Na}^+\text{influx}\} = \text{INaG} + \text{INaS} + \text{INaNa} \quad (4b)$$

$$\{\text{K}^+\text{efflux}\} = \text{EKg} + \text{EKs} \quad (5a)$$

$$\{\text{K}^+\text{influx}\} = \text{IKP} + \text{IKG} + \text{IKS} \quad (5b)$$

The species of the ion pathway and flux direction were marked by abbreviations: “P” (pump), “G” (Goldman’s channels), “NaNa” (Na^+/Na^+ exchanger), “S” (symport), “E” (efflux), “I” (influx). So, the term ENaP is Efflux Na^+ via Pump, INaG is Influx Na^+ via Goldman’s channels, ENaS is Efflux Na^+ via Symport, EKs is Efflux K via K-Symport.

2.6. Basic equations used for modeling the balance of the monovalent ion fluxes, and cell ion and water content

The basic equations were similar to the ones used for modeling of the monovalent ion distribution in red blood cells (Lew and Bookchin, 1986; Jakobsson, 1980), in proliferating cultured cells (Vereninov and Marakhova, 1986; Vereninov et al., 1995, 1997), and for analysis of the apoptotic cell volume decrease in lymphoid cells (Vereninov et al., 2004; Vereninov et al., 2006). The experiments showed that the net Na^+ and K^+ fluxes associated with changes in the ion content at apoptosis are small compared to the unidirectional fluxes. Therefore, the terms with the time derivatives in ion and water flux equations were neglected, i.e. the balanced state of cells with respect to ion and water distribution was considered:

$$[\text{Na}^+]_i + [\text{K}^+]_i - [\text{Cl}^-]_i + zA/V = 0 \quad (6)$$

$$[\text{Na}^+]_i + [\text{K}^+]_i + [\text{Cl}^-]_i + A/V = [\text{Na}^+]_o + [\text{K}^+]_o + [\text{Cl}^-]_o \quad (7)$$

$$p_1 u \{ ([\text{Na}^+]_i \exp(u) - [\text{Na}^+]_o) / (1 - \exp(u)) \} - J_{\text{PUMP}} + S_{\text{Na}} = 0 \quad (8)$$

$$p_2 u \{ ([\text{K}^+]_i \exp(u) - [\text{K}^+]_o) / (1 - \exp(u)) \} + J_{\text{PUMP}}/\gamma + S_{\text{K}} = 0 \quad (9)$$

$$p_3 u \{ ([\text{Cl}^-]_i - [\text{Cl}^-]_o \exp(u)) / (1 - \exp(u)) \} + S_{\text{Cl}} = 0 \quad (10)$$

Eqs. (6), (7) represent the electroneutrality of solutions separated by the cell membrane and osmotic equilibrium between cell and medium. $[\text{Na}^+]_o$, $[\text{K}^+]_o$, $[\text{Cl}^-]_o$ and $[\text{Na}^+]_i$, $[\text{K}^+]_i$, $[\text{Cl}^-]_i$ are external and intracellular ion concentrations in cell water, in mM; A is the intracellular content of impermeant anions, as moles per cell; z is the average charge number of these anions, taken as -1.5 ; V is the cell volume per L, taken to be equal to the water content; u is the dimensionless transmembrane electric potential difference, $u = FU/RT$, where U is the potential difference in mV. Eqs. (8)–(10) represent the balance

of the total influx and efflux across membrane for every species of ions. The first terms in Eqs. (8)–(10) are the fluxes through the channels defined as in Goldman's theory. The pump term, J_{PUMP} , in Eqs. (8), (9) was considered, unless otherwise specified, as a linear function of the intracellular Na^+ concentration:

$$J_{\text{PUMP}} = b\beta[\text{Na}^+]_i \quad (11)$$

The dimensionless parameter β ("pump rate coefficient") characterizes the intrinsic properties of the pump, whereas b represents the properties of cell influencing the effect of the pump on the intracellular ion concentration. Generally, the pump flux can be a non-linear function of intracellular Na^+ concentration (Garay and Garrahan, 1973). However, special analysis showed that taking into account non-linearity of the flux-concentration relationship does not change the main conclusions of the present paper. Coefficient γ is the stoichiometric coefficient for K^+ and Na^+ transport by the pump. The "symport" terms S_{Na} , S_{K} , S_{Cl} were defined by the next formulae:

For Na-Cl symport (NC):

$$S_{\text{Na}} = S_{\text{Cl}} = q_{\text{NC}} \left\{ 1 - \frac{([\text{Na}^+]_i [\text{Cl}^-]_i)}{([\text{Na}^+]_o [\text{Cl}^-]_o)} \right\} \quad (12a)$$

$$S_{\text{K}} = 0 \quad (12b)$$

For K-Cl symport (KCC):

$$S_{\text{Na}} = 0 \quad (13a)$$

$$S_{\text{K}} = S_{\text{Cl}} = q_{\text{KCC}} \left\{ 1 - \frac{([\text{K}^+]_i [\text{Cl}^-]_i)}{([\text{K}^+]_o [\text{Cl}^-]_o)} \right\} \quad (13b)$$

For NaK2Cl symport (NKCC):

$$S_{\text{Na}} = S_{\text{K}} = q_{\text{NKCC}} \left\{ 1 - \frac{([\text{Na}^+]_i [\text{K}^+]_i [\text{Cl}^-]_i^2)}{([\text{Na}^+]_o [\text{K}^+]_o [\text{Cl}^-]_o^2)} \right\} \quad (14a)$$

$$S_{\text{Cl}} = 2S_{\text{Na}} \quad (14b)$$

where q_{NC} , q_{KCC} and q_{NKCC} are the cation influxes representing the intrinsic properties of antiporters when the ion composition of the medium is constant. The 1:1 stoichiometry of the Na^+ and Cl^- symport follows from the 1:1 stoichiometry of the transport of H^+ and HCO_3^- out of cell that is indispensable when intracellular pH holds constant.

The solution of Eqs. (6)–(14) gives the values of five basic variables: $[\text{Na}^+]_i$, $[\text{K}^+]_i$, $[\text{Cl}^-]_i$, U and V_c and the values of ion fluxes as their derivatives. Cell volume V_c is defined as the volume per mole of impermeant anions in the cell, $V_c = V/A$.

For operation with ion fluxes, it is useful to introduce dimensionless coefficients of the integral channel permeability p_{Na} , p_{K} , p_{Cl} and dimensionless symport parameters Q_{NC} , Q_{KCC} , Q_{NKCC} according to formulae:

$$p_{\text{Na}} = p_1/b, \quad p_{\text{K}} = p_2/b, \quad p_{\text{Cl}} = p_3/b \quad (15)$$

$$Q_{\text{NC}} = q_{\text{NC}}/\{b[1\text{mM}]\}, \quad Q_{\text{KCC}} = q_{\text{KCC}}/\{b[1\text{mM}]\}, \\ Q_{\text{NKCC}} = q_{\text{NKCC}}/\{b[1\text{mM}]\} \quad (16)$$

The Equations (6)–(10) will be rearranged therefore into Eqs. (17)–(21)

$$p_{\text{Na}}u \left\{ \frac{([\text{Na}^+]_i \exp(u) - [\text{Na}^+]_o)}{(1 - \exp(u))} \right\} - \beta[\text{Na}^+]_i + f_{\text{Na}} = 0 \quad (17)$$

$$p_{\text{K}}u \left\{ \frac{([\text{K}^+]_i \exp(u) - [\text{K}^+]_o)}{(1 - \exp(u))} \right\} + \beta[\text{Na}^+]_i/\gamma + f_{\text{K}} = 0 \quad (18)$$

$$p_{\text{Cl}}u \left\{ \frac{([\text{Cl}^-]_i - [\text{Cl}^-]_o \exp(u))}{(1 - \exp(u))} \right\} + f_{\text{Cl}} = 0 \quad (19)$$

$$[\text{Cl}^-]_i = z \left\{ \frac{([\text{Na}^+]_o + [\text{K}^+]_o + [\text{Cl}^-]_o)}{(1+z)} \right. \\ \left. + \frac{([\text{Na}^+]_i + [\text{K}^+]_i)}{(1+z)} \right\} \quad (20)$$

$$(V/A) = (1+z) / \left\{ \frac{([\text{Na}^+]_o + [\text{K}^+]_o + [\text{Cl}^-]_o)}{(1+z)} - 2 \frac{([\text{Na}^+]_i + [\text{K}^+]_i)}{(1+z)} \right\} \quad (21)$$

The terms f_{Na} , f_{K} , f_{Cl} are defined by the formulae: For Na-Cl symport:

$$f_{\text{Na}} = f_{\text{Cl}} = (Q_{\text{NC}} [1\text{mM}]) \left\{ 1 - \frac{([\text{Na}^+]_i [\text{Cl}^-]_i)}{([\text{Na}^+]_o [\text{Cl}^-]_o)} \right\} \quad (22a)$$

$$f_{\text{K}} = 0 \quad (22b)$$

For K-Cl symport:

$$f_{\text{Na}} = 0 \quad (23a)$$

$$f_{\text{K}} = f_{\text{Cl}} = (Q_{\text{KCC}} [1\text{mM}]) \left\{ 1 - \frac{([\text{K}^+]_i [\text{Cl}^-]_i)}{([\text{K}^+]_o [\text{Cl}^-]_o)} \right\} \quad (23b)$$

For NKCC symport:

$$f_{\text{Na}} = f_{\text{K}} = (Q_{\text{NKCC}} [1\text{mM}]) \\ \times \left\{ 1 - \frac{([\text{Na}^+]_i [\text{K}^+]_i [\text{Cl}^-]_i^2)}{([\text{Na}^+]_o [\text{K}^+]_o [\text{Cl}^-]_o^2)} \right\} \quad (24a)$$

$$f_{\text{Cl}} = 2f_{\text{Na}} \quad (24b)$$

Dimensionless Na^+ and K^+ fluxes are defined therefore by the formulae:

$$\text{ENaP} = \beta[\text{Na}^+]_i / [\text{Na}^+]_{\text{Ref}}$$

$$\text{ENaG} = |p_{\text{Na}}u \{ [\text{Na}^+]_i \} e^u / (1 - e^u)| / [\text{Na}^+]_{\text{Ref}}$$

$$\text{INa} = \left| \left\{ -p_{\text{Na}}u [\text{Na}^+]_o / (1 - e^u) + Q_{\text{NC}} [1\text{mM}] \right\} \right| / [\text{Na}^+]_{\text{Ref}}$$

$$\text{IKP} = \{ (\beta/\gamma) [\text{Na}^+]_i \} / [\text{Na}^+]_{\text{Ref}}$$

$$\text{IKG} = | -p_{\text{K}}u \{ [\text{K}^+]_o / (1 - e^u) \} | / [\text{Na}^+]_{\text{Ref}}$$

where $[\text{Na}^+]_{\text{Ref}}$ is the intracellular Na^+ concentration at the state taken as the "reference". The numeric values in Figs. 4–6 were derived, unless otherwise specified, at $p_{\text{Na}} = 0.05$, $p_{\text{K}} = 0.5$, $p_{\text{Cl}} = 0.1$. The concentrations in the external media were taken as follows: $[\text{Na}]_o = 150$ mM, $[\text{K}]_o = 5$ mM, $[\text{Cl}]_o = 155$ mM. The stoichiometric pump coefficient γ was taken as 1.5. These values give the resting membrane potential -48 mV. For other values of resting potential the necessary value for the permeability coefficients should be somewhat changed. The solution of Eqs. (17)–(21) gives all fluxes except the equivalent Na^+/Na^+ exchange, ENaNa. The latter was obtained from the difference in total Na^+ flux and Na^+ flux via the pump observed in the experiments.

3. Results

3.1. $^{22}\text{Na}^+$, Li^+ , Rb^+ exchange and distribution in U937 cells under the balanced distribution of major ions

Fig. 1 shows the time-course of equilibration of $^{22}\text{Na}^+$, Li^+ and Rb^+ between external solution and U937 cells incubating in RPMI medium. Li^+ was used as an analogue of Na^+ for most of the transport systems except the sodium pump while Rb^+ was used as a congener of K^+ . The equilibration of monovalent cations across the plasma membrane is reached much faster in proliferating cells like U937 line than in non-proliferating differentiated cells commonly used as an object in the ion transport studies in general physiology. About 15–18% of cell Na^+ is exchanged per 1 min in U937 cells cultivated in the RPMI medium. For Li^+ and Rb^+ the values 4–6% and 0.8% per 1 min were found, respectively. The rate of exchange was dependent of the state of cell culture and in some experiments was even higher than the values above (Table 1).

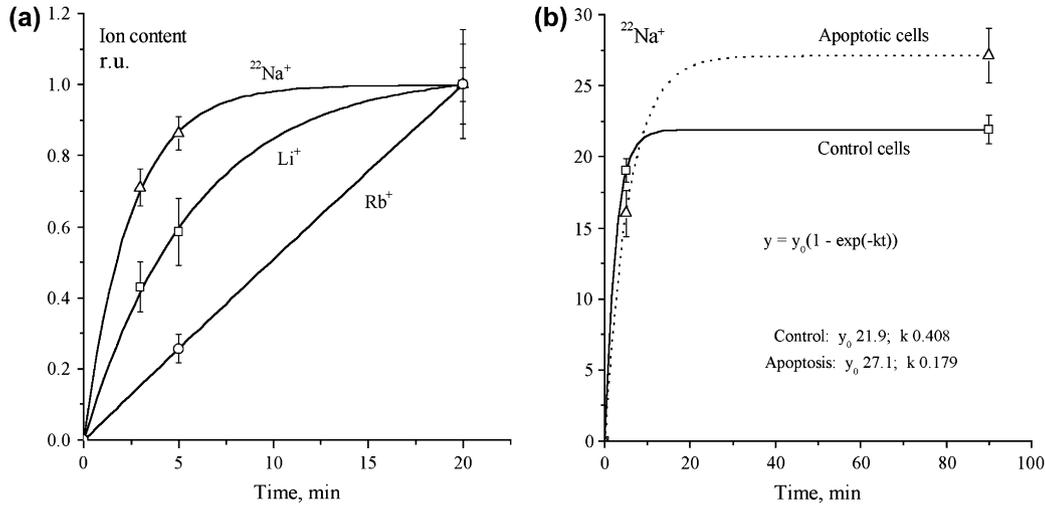


Fig. 1. The time course of $^{22}\text{Na}^+$, Li^+ and Rb^+ uptake by control (a, b) and apoptotic (b) U937 cells. Ordinate (a) $^{22}\text{Na}^+$, Li^+ and Rb^+ content, relative units (b) $^{22}\text{Na}^+$ content, 10^3 cpm mg^{-1} protein. At zero time the cells were placed into RPMI medium with $^{22}\text{Na}^+$, 5 mM Li^+ and 2.5 mM Rb^+ . The data are means \pm SE of four independent experiments, each with duplicate determinations.

Addition of 1 mM Rb^+ or 3–5 mM Li^+ for 24 h to culture of U937 cells did not appreciably modify cell proliferation. The final intracellular Rb^+/K^+ ratio differed from the ratio in the medium only as 1.14/1 ($P < 0.001$, $n = 24$). Therefore Rb^+ can be considered as a good tracer of K^+ fluxes. Distribution of Li^+ , like Na^+ , differed sharply from the distribution of Rb^+ and K^+ . However, Li^+ released from cells 2.5–3 times slower than $^{22}\text{Na}^+$. The Li^+ cell/medium ratio under the balanced state differed from the ratio for Na^+ to the same extent (Table 2).

3.2. Changes in cell ion content during apoptosis is a slow drift of a ‘‘balanced state’’

Apoptosis of U937 cells induced by 4 h incubation with 1 μM staurosporine was accompanied by a decrease in intracellular K^+ and Rb^+ by 26–37% and by an increase in intracellular Na^+ by 13–21% for 4 h (Table 1). The upper limit of

the net flux of Na^+ associated with apoptotic cell Na^+ increase can be estimated as 0.08% of the cell Na^+ content per 1 min ($0.21/240 \text{ min} = 0.0008 \text{ min}^{-1}$). This is a negligible value as compared with the unidirectional flux which is about 15–18% of cell Na^+ content per 1 min. One may conclude therefore that the apoptotic changes in cell Na^+ content are indeed a slow drift of the ‘‘balanced state’’. The same is true for the Li^+ fluxes. In the case of Rb^+ (K^+) fluxes the upper limit of the net flux and unidirectional flux can be estimated as 0.11% ($0.27/240$, see Table 1, experiments 1) and 0.8%, respectively. In fact, the apoptotic changes in cell Na^+ and K^+ content occur mostly over the first 1–2 h (Yurinskaya et al., 2005a). Therefore, the differences between the net and unidirectional fluxes are even more than what follows from the above calculations, and the apoptotic changes of the intracellular cation content should be considered as a slow drift of the balanced state.

Table 1
 Na^+ , Li^+ , K^+ , Rb^+ content and the efflux rate constants in normal and apoptotic U937 cells

Cation	Expt.	Ion content, $\mu\text{mol g}^{-1}$			$*k_{io}$, min^{-1}			$**k_{io}$, min^{-1}		
		Control	Apoptosis	Ratio A/C	Control	Apoptosis	$*k^C/*k^A$	Control	Apoptosis	$**k^C/**k^A$
Na^+	1	228 \pm 6 (25)	258 \pm 6 (29)	1.13	0.152 \pm 0.009 (16)	0.060 \pm 0.007 (12)	2.53	0.175*	0.178	2.36
	2	283 \pm 12 (22)	337 \pm 14 (18)	1.19						
	3	268 \pm 12 (19)	323 \pm 23 (18)	1.21						
Li^+	1	26.6 \pm 1.0 (27)	25.4 \pm 1.0 (29)	0.95	0.036 \pm 0.002 (20)	0.027 \pm 0.001 (18)	1.33	0.044*	0.031	1.42
	2	24.7 \pm 0.8 (34)	25.5 \pm 0.5 (10)	1.03						
	3	21.2 \pm 1.0 (27)								
K^+	1	892 \pm 27 (27)	658 \pm 28 (29)	0.74	0.0077 \pm 0.0008 (20)	0.0067 \pm 0.0010 (20)	1.15			
	2	808 \pm 25 (20)	554 \pm 28 (20)	0.69						
	3	820 \pm 15 (17)	517 \pm 20 (18)	0.63						
Rb^+	1	178 \pm 3 (24)	129 \pm 3 (24)	0.73						

Cells were loaded by Rb^+ and Li^+ in RPMI media with 5 mM LiCl and 1 mM RbCl for 24 h (experiments 1) or with 3–5 mM LiCl for 1.5 h (experiments 2, 3, the values recalculated for 5 mM), then treated with 1 μM STS for 4 h in the same medium. The loading of cell by $^{22}\text{Na}^+$ was performed during the last 1.5 h in the medium with STS and additionally $^{22}\text{Na}^+$. $*k_{io}$, rate constants for the loss of ions calculated by formula: $*k_{io} = -1/t \ln(y_t/y_0)$, where y_t/y_0 is a ratio of the residual ion quantity in cells to the sum of that and the quantity appeared in the medium; $**k_{io}$, rate constants obtained by fitting uptake of ions with the function $y_t = y_0(1 - \exp(-**k_{io} t))$ for the time points 10 and 90 min (values marked as *) or for the time points 3, 5, 20 min (values marked as **). The data are means \pm SE. The number of determinations, n , indicated in parentheses. Experiments 1, 2 and 3 were performed in different years.

Table 2
Li⁺/Na⁺ discrimination by U937 cells

LiCl, mM (medium)	Cells	Li _i ⁺ /Na _i ⁺	Li _o ⁺ /Na _o ⁺	(Li _i ⁺ · Na _o ⁺)/(Li _o ⁺ · Na _i ⁺)		n
				Control	Apoptosis	
3 mM, 24 h	Control	0.074 ± 0.003	0.024 ± 0.001	3.1		8
5 mM, 24 h	Control	0.120 ± 0.003	0.040 ± 0.001	3.0		60
	Apoptotic	0.099 ± 0.003	0.040 ± 0.001		2.5	29
5 mM, 1.5 h	Control	0.100 ± 0.005	0.041 ± 0.001	2.4		34
	Apoptotic	0.078 ± 0.004	0.042 ± 0.002		1.9	16
7.5 mM, 1.5 h	Control	0.14 ± 0.01	0.067 ± 0.006	2.1		10
	Apoptotic	0.11 ± 0.005	0.066 ± 0.006		1.6	10

Cells were incubated in RPMI medium with LiCl for 24 h and with 1 μM STS for the last 4 h or in RPMI medium with 1 μM STS for 4 h and with LiCl for the last 1.5 h. The data are means ± SE.

3.3. Changes in the efflux and influx rate constants and the shift in ion balance in apoptotic cells. Arguments for Na⁺/Na⁺ and Li⁺/Li⁺ exchange

The efflux rate constant for Na⁺ in apoptotic cells was lower than the one in the control cells by approximately 2.5 times (Table 1), whereas cell Na⁺ content under the steady state was higher only by 1.13–1.21 times. Thus, the decrease in the Na⁺ efflux under apoptosis was balanced to a large extent by a decrease in Na⁺ influx (Eq. (3) in Section 2). This suggests the existence of an equivalent Na⁺/Na⁺ exchange, which is reduced under apoptosis. Similarly, the efflux rate constant for Li⁺ in the apoptotic cells decreased by 1.3–1.5 times, whereas cell Li⁺ content under the balanced state remained constant. This is an indication that a decrease in the efflux rate constant for this ion was related to a decrease in the Li⁺/Li⁺ exchange.

The Rb⁺ influx measured at 2.5 mM external Rb⁺ was found to be 1.13 ± 0.01 in apoptotic cells vs 2.02 ± 0.02 μmol g⁻¹ min⁻¹ in the normal cells, i.e. it was lower in apoptotic cells by 1.8 fold. The efflux rate constant for Rb⁺ was lower in apoptotic cells only by 1.15 fold (a significant difference, *P* = 0.015, Table 1). No significant differences in

the efflux rate constant for Rb⁺ in the apoptotic and normal cells were found in other separate series of experiments (data not shown). We may conclude that the apoptotic shift in the Rb⁺ (K⁺) distribution, i.e. a decrease of cell Rb⁺ (K⁺) content, is due mostly to a decrease in Rb⁺ (K⁺) influx, but not to its efflux.

3.4. Recognition of the ion pathways by using inhibitors

Ouabain suppressed for the most part Rb⁺ influx in both the normal and apoptotic cells. The ouabain-inhibitable component of the Rb⁺ influx should be qualified as a pump influx. The pumped Rb⁺ influx over the first 10 min time interval was in normal and apoptotic cells on average 1.62 ± 0.02 and 0.67 ± 0.04 μmol g⁻¹ min⁻¹, or 80% and 59% of the total influx, respectively (Fig. 2a). In the time interval between 10–30 min, the gain of Rb⁺ in non-apoptotic cells treated with ouabain remained very low while in apoptotic cells ouabain-resistant Rb gain increased and appeared to be significantly higher than in non-apoptotic cells treated with ouabain (7.5 ± 0.7 and 12.2 ± 1.0 μmol g⁻¹ at the 30 min time-point compared with 4.0 ± 0.2 and 4.6 ± 0.3 μmol g⁻¹ at the 10 min time-point. The increase in the ouabain-resistant Rb⁺

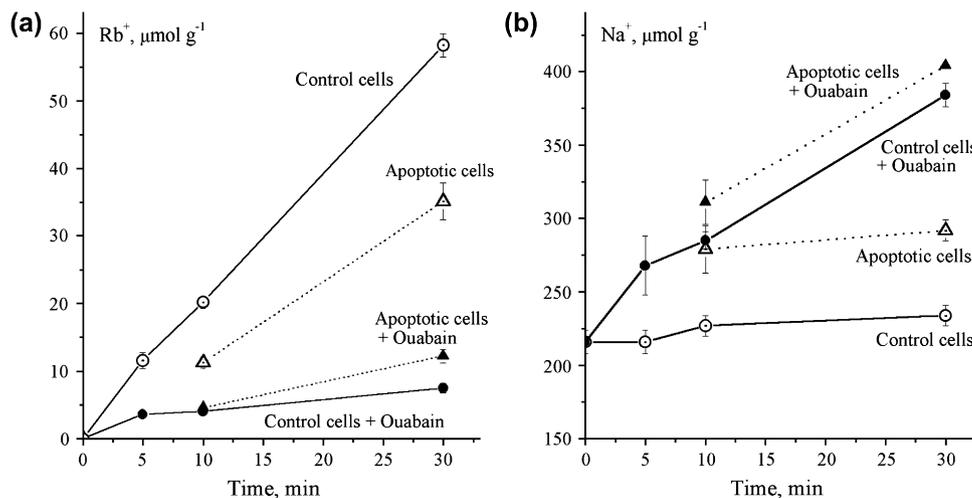


Fig. 2. Effect of ouabain on Rb⁺ uptake (a) and on cell Na⁺ content (b) in apoptotic and control U937 cells. Cells were incubated for 4 h in RPMI medium with (dotted lines) or without (solid lines) 1 μM STS and then for 5, 10, 30 min in the same medium with addition of 2.5 mM RbCl with (solid symbols) or without (open symbols) of 0.1 mM ouabain. The data are means ± SE of 7–40 determinations for control cells and of 6–12 determinations for apoptotic cells.

influx after incubation of the apoptotic cells with ouabain for 30 min should be taken into account when the pump component is determined as a difference between Rb^+ uptake with and without ouabain for 30 min. In any case, it is a decrease in the influx Rb^+ (K^+) via the pump that is responsible for the most part of the decrease in the total Rb^+ (K^+) influx during apoptosis. The ouabain-resistant component of Rb^+ influx was higher in apoptotic cells by 1.13–1.63 fold.

Treatment of cells with ouabain for 30 min was followed by a twofold increase in cell Na^+ content (Fig. 2b). There was no indication that a new balanced state could be reached in this case. The ratio of the increment in Na^+ content to the decrement in K^+ content in cells treated with ouabain for 5–10 min was in the range of 1.2–1.8, which is typical for the Na^+ , K^+ -ATPase pump (Table 3). Increment in the intracellular Na^+ content caused by ouabain was lower in the apoptotic vs. control cells. This corresponds to the reduced pump activity in the apoptotic cells found by measuring the ouabain-inhibitable Rb^+ uptake.

Fig. 3 shows the effect of ouabain and a mixture of DMA with DIDS (DD) on the $^{22}\text{Na}^+$ and Li^+ efflux rate-constants measured simultaneously in the same cells. The efflux rate-constant for Na^+ was decreased by 10 min of ouabain treatment by about 25% compared with control U937 cells, whereas in the apoptotic cells the ouabain-inhibitable component in the Na^+ efflux rate constant was lower and only detected with difficulty. These data confirm once again that the pumping of Na^+ out of the apoptotic cells is reduced. The total unidirectional Na^+ efflux calculated as a product of the efflux rate constant and the intracellular Na^+ content obtained by flame photometry in these experiments was on average 42 and 24 $\mu\text{mol min}^{-1} \text{g}^{-1}$ for normal and apoptotic U937 cells, respectively. The Na^+ efflux via the pump calculated as a difference in the Na^+ efflux in cells treated and untreated with ouabain for 10 min (ENaP) was 9.6 and 4.4 $\mu\text{mol g}^{-1} \text{min}^{-1}$ in the non-apoptotic and apoptotic cells, respectively.

To establish which pathways provide large ouabain-resistant Na^+ efflux, we studied the effects of DMA, DIDS, and bumetanide as the inhibitors of Na^+/H^+ , $\text{Cl}^-/\text{HCO}_3^-$ antiporters and NaK_2Cl symporter, respectively. The effect of bumetanide on the efflux of Na^+ appeared to be small and insignificant (data not shown). It should be noted, however, that a small but statistically significant decrease in Rb^+ influx caused by

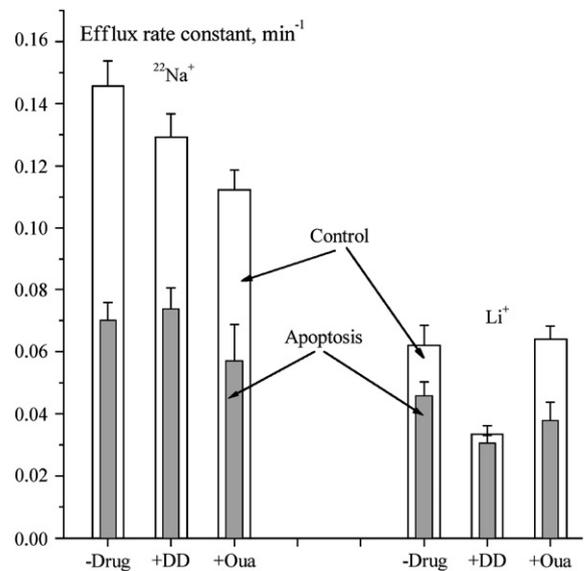


Fig. 3. Effect of DMA + DIDS (DD) and ouabain (Oua) on $^{22}\text{Na}^+$ and Li^+ rate constants in control and apoptotic U937 cells. Cells were incubated for 4 h in the RPMI medium with (grey columns) or without (white columns) of 1 μM STS and for the last 1.5 h with ^{22}Na and 5 mM LiCl. Then the cells were transferred for 10 min into the ^{22}Na and Li^+ -free medium with or without drugs: 0.05 mM DMA + 0.5 mM DIDS or 0.1 mM ouabain. Rate constant was calculated by formula $k = -(1/t)\ln(y_t/y_0)$, where y_t/y_0 is the ratio of the residual ion quantities in cells to the sum of that and the ions appeared in the medium. The data are means \pm SE of 9 independent experiments for control cells and of 5 experiments for apoptotic cells, each with duplicate determinations.

bumetanide occurred. Therefore, the cells were in general sensitive to bumetanide. The effect of a mixture of DMA + DIDS (DD) on the efflux rate constant for Na^+ was small and insignificant both in the normal and apoptotic cells in the data presented in Fig. 3, although a slight but significant inhibitory effect of DD was sometimes observed in other experiments. No significant effect of 0.1 mM DIOA (as inhibitor of KCl symport) was found in short-term measurements of Rb^+ fluxes. The treatment of U937 cell with DIOA for 0.5–4 h was followed by a decrease in the intracellular K^+/Na^+ ratio.

There was no effect of ouabain on the Li^+ efflux in the normal or apoptotic cells. The fact that the Li^+ distribution under the balanced state is far from the electrochemical equilibrium and that Li^+ is not pumped out of the cell by the sodium pump indicates that some part of the Li^+ efflux should be involved in a secondary active transport produced by the Li^+/Na^+ or Li^+/H^+ antiport (Grinstein et al., 1984). Surprisingly, the Li^+ efflux was only partially inhibited by DD. The mixture DD decreased Li^+ efflux by $\sim 51\%$ and 33% in the normal and apoptotic cells, respectively. Insensitivity of the significant part of the Li^+ efflux to DD corresponds to the DD insensitivity of the most part of the Na^+ efflux. These components of the Na^+ and Li^+ fluxes resemble the amiloride-insensitive Na^+/Na^+ exchange observed in rat thymocytes (Grinstein et al., 1984), and amiloride-insensitive Na^+/Li^+ exchange shown in PS 200 hamster fibroblast cell line transfected with amiloride-insensitive isoform NHE (DNHE-1) (Zerbini et al., 2003).

Table 3

The shift in the intracellular Na^+ and ($\text{Rb}^+ + \text{K}^+$) content caused by ouabain for 5, 10, 30 min in control and apoptotic U937 cells

Time, min	Control cells		Apoptotic cells	
	Na^+	$\text{Rb}^+ + \text{K}^+$	Na^+	$\text{Rb}^+ + \text{K}^+$
5	+54 \pm 10 (7)	-30 \pm 5 (9)		
10	+60 \pm 5 (45)	-47 \pm 3 (16)	+30 \pm 11 (11)	-22 \pm 2 (16)
30	+150 \pm 6 (35)	-179 \pm 6 (28)	+108 \pm 12 (9)	-76 \pm 8 (6)

The data are differences between the Na^+ and ($\text{Rb}^+ + \text{K}^+$) contents after incubation in RPMI medium contained 2.5 mM RbCl with and without 0.1 mM ouabain, $\mu\text{mol g}^{-1}$. The data are means \pm SE. The number of determinations, n , is indicated in parentheses.

3.5. Using the model for analysis of ion fluxes and water balance in U937 cells

The balance of the monovalent ion fluxes across the cell membrane depends, generally, on the multiple factors that can vary in multiple combinations. Analysis of this complicated multiparametric system can be simplified in some specific cases. In the present study, the intracellular K^+/Na^+ ratio and the relationship between the “pump” and “channel” components of the total K^+ influx (IKG/IKP) were chosen as the primary criteria in order to select the physiologically significant parameters. This way it was easier to obtain the “rigid” model available for predictions and validation by comparison of the calculated and experimental values. The state with the K^+/Na^+ ratio of 4.5 and IKG/IKP of 0.24 was taken as a “reference”. Our modeling was focused on the question of what kind of changes in ion transporters could cause transition of cells from the reference balanced state 1 to the new balanced state 2. This new state is associated with (1) a drop of the K^+/Na^+ ratio to 3, (2) a decrease in Na^+ and K^+ fluxes via the pump by a factor 2.4, (3) an increase in IKG by 15%, and (4) a loss of cell water by 22%, that is near the values observed in experiments with the normal and apoptotic U937 cells. Therefore, analysis of the model was performed within and near this range.

Fig. 4 demonstrates the relationships between the pump rate constant β and major characteristics of ion balance when the system includes solely the pump and electroconductive

channels (solid line without symbols), and when additionally NaKCl (NKCC), K-Cl (KCC) and Na-Cl (NC) symports occur in parallel with the ion pumping and electrodiffusion through the channels (curves with symbols). Decrease in the pump rate coefficient leads to a decrease in intracellular K^+/Na^+ ratio and transmembrane electrical potential difference U in all cases. NKCC and NC symports significantly increase cell water content whereas KCC, in contrast, decreases it. Decrease of β in the cell model with symports NC and KCC is followed by an increase in cell water content, as in the model without symports. In contrast, a decrease in cell volume is observed due to a decrease of β up to the value 0.2 in the model with NKCC symport (open circles). It is important that the significant changes in cell water content due to NC and NKCC symports occur even when their share in the total Na^+ and K^+ fluxes is small, e.g. at $Q_{NC} = 0.56$ when the symport components comprise 2% of the Na^+ pump efflux. The effect of such small symport fluxes on the K^+/Na^+ ratio, membrane potential and the pump fluxes is negligible. The Na^+ efflux and K^+ influx via the pump, ENaP and IKP, are reduced with a decrease of β in the “physiological” range of K^+/Na^+ ratios much more slowly than β because of the parallel increase in $[Na^+]_i$. Moreover, in this model it is not possible to obtain the reduction of the pump fluxes by a factor 2.4 in parallel with a decrease in K^+/Na^+ ratio from 4.5 to 3.0. Fig. 4e demonstrates that the Na^+ efflux through channels, ENaG, in “physiological” range of K^+/Na^+ ratios is very small in compare with the total Na^+ flux and with ENaP. Therefore,

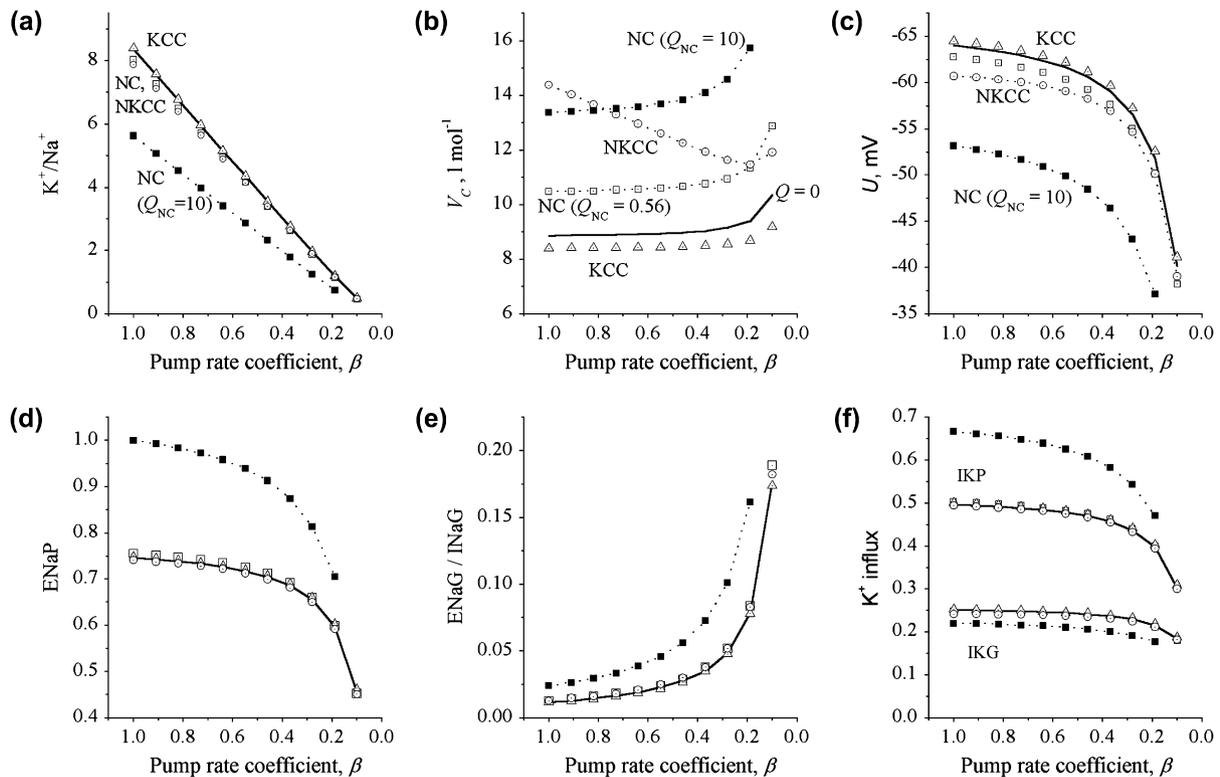


Fig. 4. Dependence of intracellular K^+/Na^+ ratio, cell water content, resting membrane potential and cation fluxes on the pump rate coefficient calculated for cell model without symports (solid line) and with symports NaK2Cl (NKCC, circles), K-Cl (KCC, triangles) and Na-Cl (NC, open squares at $Q_{NC} = 0.56$, solid squares at $Q_{NC} = 10$). pCl was taken as 0.01 except the case when Q_{NC} was 10 ($pCl = 0.1$), $Q_{KCC} = 0.56$, $Q_{NKCC} = 2.8$, $pNa = 0.05$, $pK = 0.5$. Fluxes were normalized as indicated in Section 2 at Na in reference state - 28. For comparison of symport influx with ENaP the parameters Q should be divided by a factor 28.

practically all Na^+ efflux should be equal to ENaP. That does not correspond to the experimental findings.

Several variants of changes in ion pathways other than the sodium pump degradation were examined to find out which pathways could be responsible for transition of cells from

the “normal” state 1 to the “apoptotic” state 2. Since there is a widespread assumption that K^+ channels opening leads to the apoptotic changes in ion and water balance (Burg et al., 2006; Lang et al., 2006) it was interesting to check this hypothesis by modeling.

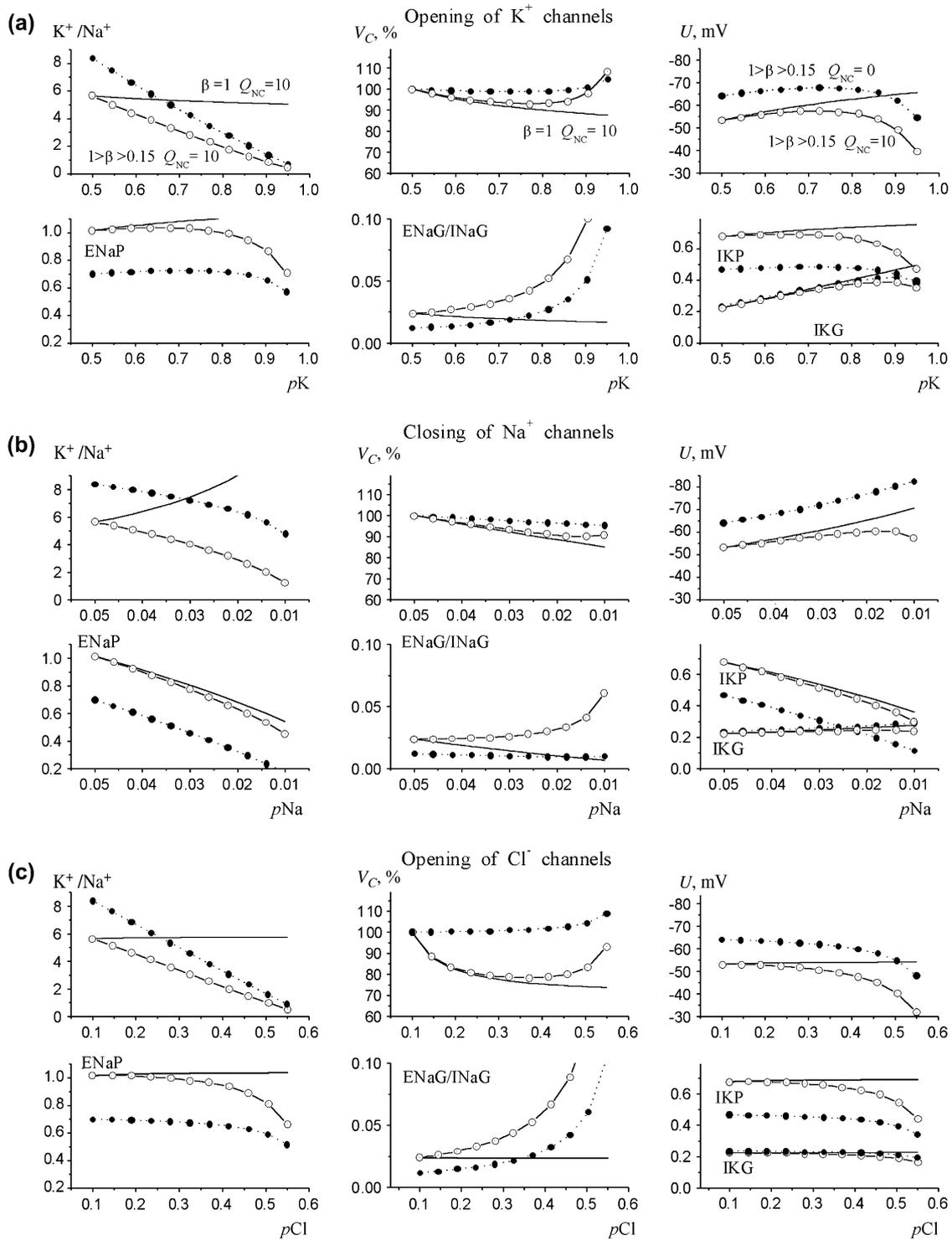


Fig. 5. Dependence of intracellular K^+/Na^+ ratio, cell water content, resting membrane potential and cation fluxes on the integral permeability of K^+ , Na^+ , and Cl^- channels calculated for cell model under the constant kinetic parameters for the pump and Na-Cl symport (solid line without symbols) and under decreasing pump rate coefficient (circles) in the presence (open circles) or absence of Na-Cl symport (solid circles). Invariable parameters were as follows: (a) $p\text{Na} = 0.05$, $p\text{Cl} = 0.1$, $Q_{\text{NC}} = 0$ or $Q_{\text{NC}} = 10$; (b) $p\text{K} = 0.5$, $p\text{Cl} = 0.1$, $Q_{\text{NC}} = 0$ or $Q_{\text{NC}} = 10$; (c) $p\text{Na} = 0.05$, $p\text{K} = 0.5$, $Q_{\text{NC}} = 0$ or $Q_{\text{NC}} = 10$. For other details see Section 2.

Fig. 5 shows that K^+ channel opening with the constant kinetic coefficients for the pump and NC symport should be accompanied by (1) a relatively small cell volume reduction, (2) an increase in the resting membrane potential, (3) a small decrease in K^+/Na^+ ratio, (4) an increase in the pump fluxes (solid line without symbols). Hence, K^+ channel opening alone could not explain the transition of cells to the apoptotic state. K^+ channel opening in parallel with a decrease in the pump rate coefficient can decrease K^+/Na^+ ratio and cell volume, but not the pump fluxes to the required extent. Cl^- channel opening is a powerful regulator of cell volume when moderate Na-Cl symport is operating and gives the necessary decrease in K^+/Na^+ ratio but does not cause the required reduction in the pump fluxes.

When Na^+ channels close alone the cell volume reduction is accompanied by the increase in K^+/Na^+ ratio. When combined with the pump degradation the Na^+ channels closing can be accompanied by both K^+/Na^+ ratio and cell water content reduction together with an increase of the resting membrane potential. In this case the pump fluxes are reduced more significantly than in the case of the combined decrease in the pump rate coefficient and K^+ or Cl^- channels opening. However, Na^+ channels closure and decrease in pump activity are not sufficient alone to cause the expected cell volume reduction.

Analysis of the effects caused by separate pathways lead to the conclusion that these are the changes in more than one ion pathway that are responsible for the transition from the “normal” ion and water balance at the state 1 to the “apoptotic”

balanced state 2. The model in Fig. 6 – providing a decrease in Na^+ and K^+ pumping and in Na-Cl symport with parallel closing Na^+ channels (decrease of p_{Na} by about factor 2) – gives a satisfactory approximation. The main characteristics of ion balance are given in this case as a function of the fluxes of K^+ (influx) or Na^+ (efflux) via pump, φ_{pump} . Direct use of the pump flux as a parameter enables us to establish relationships free of the assumption that the pump flux depends on the Na^+ and K^+ concentrations inside and outside of the cell. The relationships between φ_{pump} and the apparent value of the coefficient β in the equivalent “linear” model for different cases including the nonlinear model of Garay and Garrahan (1973) are shown in Fig. 6e (the curves for the nonlinear model are shown by dotted lines without symbols). The flux φ_{pump} is the Na^+ pump efflux normalized to the value in the state 1 (“normal” cells), which is equal to 25.8, according to calculations at the following parameters: $Q_{NC} = 10$, $p_{Na} = 0.05$, $pK = 0.5$, $\beta = 0.82$. The K^+/Na^+ ratio is equal to 4.5 at these parameters. When a drop in K^+/Na^+ is preset, the required decrease in the pump fluxes can be obtained at several combinations of parameters related to the Na-Cl symport and Na^+ , K^+ channel permeability (variation of the permeability of Cl^- channels is not shown because opening of Cl^- channels acts approximately as an attenuator of the Na-Cl symport). For example, combinations $Q_{NC} = 3$, $p_{Na} = 0.022$, $pK = 0.46$ (Fig. 6a, solid triangles), and $Q_{NC} = 3$, $p_{Na} = 0.022$, $pK = 0.5$ (open triangles) yield a similar relationship between K^+/Na^+ ratio and changes in pump fluxes. It is important that these combinations appear not to be equivalent if other ion

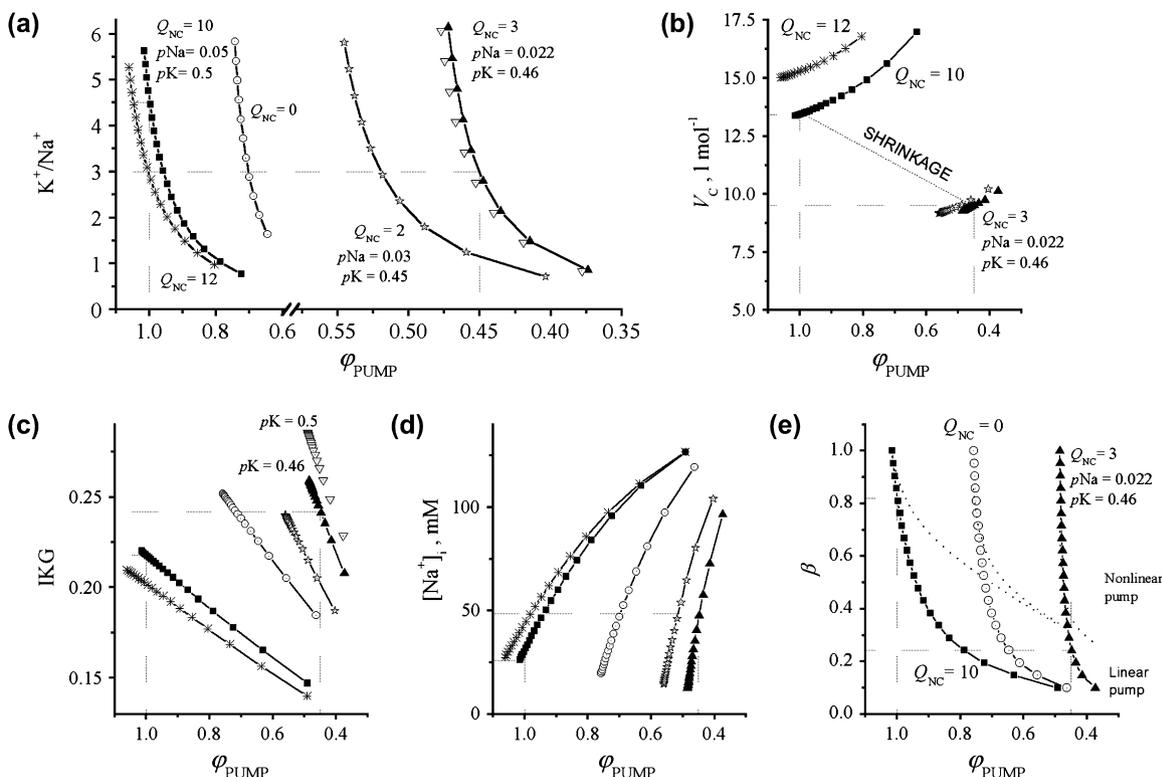


Fig. 6. Relationships between the Na^+ pump efflux and the major characteristics of the ion and water balance for the cell model with the pump, Na-Cl symport and Na^+ , K^+ , and Cl^- channels under the different values of Na-Cl symport and integral permeability of Na^+ , K^+ , and Cl^- channels. Fluxes were normalized to the value of Na^+ pump efflux at the reference state. For other details see Section 2.

balance characteristics are taken into account, e.g. relationships between changes in pump fluxes and values of the K^+ influx via channels. Therefore, correct checking of the model requires the study of the necessary number of characteristics of ion balance uniquely determined the system. Modeling helps to find the characteristics that are critical in the situation considered.

Matching of the model and the experimentally observed changes in ion and water balance in U937 cells at apoptosis (transition from the state 1 to the state 2) shows that the decrease in the Na-Cl symport is an indispensable requirement to achieve simultaneously all the complex of changes in ion and water balance observed in these cells. The second important conclusion is that a relatively small decrease in the Na-Cl symport is sufficient to cause the observed decrease in the cell water content. Since the Na-Cl flux is small, it is hardly detected by direct measurement of flux. The difficulties rise if this flux is not blocked by specific inhibitors, as it occurs in U937 cells. The significance of modeling in this case is obvious.

3.6. Balance of ion fluxes across plasma membrane of U937 cells

Eqs. (17)–(21) describe only the net fluxes via all ion pathways existing in the cell membrane. Ion exchange with a stoichiometry of 1:1, as Na^+/Na^+ exchange, does not affect ion distribution across the membrane, transmembrane electrical potential difference and cell water balance, but its contribution to the total flux is very significant. This can be seen in U937 cells. The measured total Na^+ efflux, as well as influx under the balanced Na^+ distribution was $42 \pm 2 \mu\text{mol g}^{-1} \text{min}^{-1}$. The pump component of Na^+ efflux (ENaP) was $9.6 \pm 2.0 \mu\text{mol g}^{-1} \text{min}^{-1}$. The question is, what ion pathway can be responsible for the large ouabain-resistant component of Na^+ efflux that accounts for 70–75% of the total Na^+ flux? Analysis of the model shows that it cannot be attributed to the Na^+ efflux through the channels for the following reason. The upper limit of the channel component of the Na^+ efflux is determined by Ussing's formula for electrodiffusion (Ussing, 1949; Sten-Knudsen and Ussing, 1981):

$$\text{ENaG}/\text{INaG} = \left\{ \frac{[Na^+]_i}{[Na^+]_o} \right\} \exp[FU/RT] \quad (25)$$

where ENaG and INaG are outward and inward Na^+ fluxes due to electrodiffusion through ion channels, U is the transmembrane electrical potential difference, $[Na^+]_i$ and $[Na^+]_o$ are intracellular and external Na^+ concentrations. At $\{[Na^+]_i/[Na^+]_o\} = 30 \text{ mM}/140 \text{ mM}$ (Yurinskaya et al., 2005) and if it is taken that $U = -40 \div -65 \text{ mV}$, the ratio of fluxes should be equal to 0.05–0.02.

The values of the Ussing's flux ratio for different states of the cell model are shown in Figs. 4 and 5. Therefore, even if it is assumed that all Na^+ influx is electrodiffusion through Na^+ channels, only 2–5% of the total Na^+ efflux could be related to the Na^+ efflux via channels and no less than 70% of the Na^+ efflux should be attributed to the ion pathway other than the pump and channels. Most importantly, it should be related to the pathway with zero net Na^+ flux which is not considered in the Eqs. (17)–(21) written for the net fluxes. This can only be the equivalent Na^+/Na^+ exchange. This conclusion is confirmed independently by the experimental finding that the transition to the apoptotic ion balance is accompanied by simultaneous proportional changes in the efflux and influx rate constants for Na^+ and Li^+ . Once the equivalent Na^+/Na^+ exchange is stated, the channel component in the Na^+ influx should be less than the total flux by a value of the Na^+ exchange component. Therefore, only 25–30% of the Na^+ influx is left for the channel Na^+ influx. Correspondingly, the channel component in the Na^+ efflux should be <1%. Finally, the balance of unidirectional Na^+ and K^+ fluxes via the major ion pathways in the plasma membrane of U937 cells looks like that presented in Table 4.

4. Discussion

Our model of cell ion and water balance was adapted specifically for analysis of the unidirectional ion fluxes. Using proliferating cultured cells with rapid exchange of the monovalent ions across plasma membrane enables one to study the different balanced states of the same cells. We compared U937 cells under normal culture and at apoptosis induced by staurosporine. The balanced state with respect to cell water

Table 4
Major components of Na^+ and K^+ unidirectional fluxes in normal and apoptotic U937 cells

Ion pathway	Na^+ fluxes				K^+ fluxes			
	Normal cells		Apoptotic cells		Normal cells		Apoptotic cells	
	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx
Pump	0.23 (9.6)	—	0.106 (4.4)	—	—	0.154 (5.4)	—	0.07 (2.2)
Channels	0.004	0.16	0.002	0.082	0.205	0.051 (1.3)	0.127	0.057 (1.5)
NaCl symport	0.007	0.089	0.001	0.027	—	—	—	—
Na/Na exchange	0.76	0.76	0.46	0.46	—	—	—	—
All (total flux)	1 (42)	1	0.57 (24)	0.57	0.205	0.205 (6.7)	0.127	0.127 (3.7)

Values of fluxes were calculated using the mathematical model at $Q_{NC} = 10$, $pCl = 0.1$, $pNa = 0.05$, $pK = 0.5$, $\beta = 0.82$ for "Normal cells" ("reference" state 1) and at $Q_{NC} = 3$, $pCl = 0.1$, $pNa = 0.0226$, $pK = 0.47$, $\beta = 0.24$ for "Apoptotic cells" (state 2) and normalized to the total flux of Na^+ at the reference state. The experimental values in parantheses are given in $\mu\text{mol min}^{-1} \text{g}^{-1}$ of cell protein. The Na^+ fluxes were obtained by using ^{22}Na as a tracer, the K^+ fluxes by measurement of Rb^+ fluxes.

content and distribution of Na^+ and K^+ across plasma membrane was justified by comparison of the rate of ion exchange (measurement of unidirectional fluxes) and the rate of alteration of intracellular Na^+ and K^+ content (estimation of net fluxes). This permits the use of the balance equations (17)–(21). Besides, the sodium pump and Na^+ , K^+ , and Cl^- channels, the model accounts for some other widespread transporters, the electroneutral symporter carrying Na^+ , K^+ and 2Cl^- (NKCC), the K-Cl symporter (KCC) and the complex of Na^+/H^+ with $\text{Cl}^-/\text{HCO}_3^-$ antiporters operating as the Na-Cl symporter. In the detailed analysis of the model the transporters NKCC and KCC were excluded because of the small effect of their specific inhibitors, bumetanide and DIOA, on the monovalent ion fluxes in studied U937 cells. This reduced the amount of parameters and number of the variants required to be analyzed.

The study of U937 cells showed that both modeling and experimentation are required to understand the mechanisms maintaining cellular ion and water balance. The values of the decrease in cell water content and intracellular K^+/Na^+ ratio, and changes in the total and pump fluxes of Na^+ and K^+ , were obtained in our experiments herein, as also in Yurinskaya et al. (2005). The rate constants both for the efflux and influx of Na^+ and Li^+ change significantly and to the same extent, whereas intracellular Na^+ and Li^+ contents remain constant. Hence, the Na^+/Na^+ and Li^+/Li^+ equivalent exchange takes place. On the other hand, the model shows (1) that fluxes of Na^+ into and out of cell cannot be balanced if only Na^+ channels and the sodium pump operate; (2) that without Na^+/Na^+ exchange the pump efflux of Na^+ should be practically equal to the total efflux, whereas in fact the pump flux accounts for only 23% of the total efflux; (3) that NaCl symport gives a clue to the role in shifting the water balance in cell shrinkage despite the decrease in Na^+ and K^+ pumping and also that a decrease in Na^+ flux involved in NaCl symport is too small to be detected easily by measurement of fluxes; (4) that about twofold decrease in integral Na^+ channel permeability is required to obtain the observed balance of fluxes at apoptosis when the pump Na^+ efflux decreases by a factor 2.4.

The important point in the calculation of the balance of Na^+ fluxes was the estimation of the channel component in the Na^+ efflux that is based on the principle of independence of the forward and backward fluxes of ions at electrodiffusion. Several cases are known where this principle does not hold. One of them is in “single file” diffusion (Hodgkin and Keynes, 1955; DeFelice et al., 2001). Could a similar phenomenon explain the large ouabain-resistant component of the Na^+ efflux on the assumption that it is the Na^+ efflux via channels? The unidirectional ion fluxes through the cell membrane are expressed according to the Goldman’s theory of the “independent” ion electrodiffusion by the formulas $\text{INaG} = p\text{Na}u[\text{Na}]_i/(\exp(u) - 1)$ and $\text{ENaG} = p\text{Na}u[\text{Na}]_o\exp(u)/(1 - \exp(u))$ for inward and outward ion movement, respectively. Independent opposite movement of ions through membrane means that the permeability coefficient for both fluxes is the same. This gives Ussing’s formula, Eq. (25). The case of non-independent inward and outward ion fluxes is equivalent to the Ussing relation with the distinct “permeability coefficients” for inward and outward fluxes:

$$\{\text{ENaG}/\text{INaG}\} = (P_{\text{outward}}/P_{\text{inward}}) \times ([\text{Na}]_i/[\text{Na}]_o)\exp(u) \quad (26)$$

To attribute the large ouabain-resistant Na^+ efflux in U937 cells to the movement of Na^+ through the channels it should be assumed that P_{outward} is 20–50 times higher than P_{inward} . The observed deviation from the Ussing relationship has the opposite sign compared to the single file phenomenon. In our case the permeability coefficient for the Na^+ flux against its gradient should be higher than that for the flux down gradient, whereas in the single file phenomenon the opposite deviation occurs. Hence, “non-independent” inward and outward movement of Na^+ via channels cannot explain the results observed in U937 cells.

It would be interesting to know the resting membrane potential in U937 cells at the normal and apoptotic states, because the model calculation predicts the uniquely determined values. However, we have no data yet on cell membrane potential and thus cannot discuss this issue.

Acknowledgements

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