ISSN 1990-519X, Cell and Tissue Biology, 2010, Vol. 4, No. 5, pp. 464–470. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © A.A. Rubashkin, V.E. Yurinskaya, A.A. Vereninov, 2010, published in Tsitologiya, Vol. 52, No. 7, 2010, pp. 568–573.

Calculations of K^+, Na^+, and Cl^- Fluxes across Cell Membrane with Na^+/K^+ Pump, NKCC, NC Cotransport and Ionic Channels with Non-Goldman Rectification in K^+-Channels: Normal and Apoptotic Cells[1]

A. A. Rubashkin, V. E. Yurinskaya, and A. A. Vereninov*

Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia

**e-mail: verenino@mail.ru*

Received December 1, 2009

Abstract—The balance of K^+, Na^+, and Cl^- fluxes across the cell membrane with the Na^+/K^+ pump, ion channels, and $Na^+K^+2Cl^-$ (NKCC) and $Na^+–Cl^-$ (NC) cotransport was calculated to determine the mechanism of cell shrinkage in apoptosis. It is shown that all unidirectional K^+, Na^+, and Cl^- fluxes; the ion channel permeability; and the membrane potential can be found using the principle of the flux balance if the following experimental data are known: K^+, Na^+, and Cl^- concentrations in cell water; total Cl^- flux; total K^+ influx; and the ouabain-inhibited pump component of the $Rb^+(K^+)$ influx. The change in different ionic pathways during apoptosis was estimated by calculations based on the data reported in the preceded paper (Yurinskaya et al., 2010). It is found that cell shrinkage and the shift in ion balance in U937 cells induced to apoptosis with 1 μM staurosporine occur due to the coupling of reduced pump activity with a decrease in the integral permeability of Na^+ channels, whereas K^+ and Cl^- channel permeability remains almost unchanged. Calculations show that only a small part of the total fluxes of K^+, Na^+, and Cl^- account for the fluxes mediated by NKCC and NC cotransporters. Despite the importance of cotransport fluxes for maintaining the nonequilibrium steady-state distribution of Cl^-, they cannot play a significant role in apoptotic cell shrinkage because of their minority and cannot be revealed by inhibitors.

Key words: Cl^- flux, ion channel, ion transporter, cell ion and water balance, apoptosis.

DOI: 10.1134/S1990519X10050093

There are few papers on the systemic analysis of the ionic and water balance in the cell compared to the numerous reports on various channels and transporters. However, without systemic analysis, it is impossible to understand how fluxes mediated by various channels and carriers influence ionic homeostasis in the whole cell. The systemic analysis of the ion homeostasis is important, in particular with regard to apoptosis. The regulation of apoptosis by monovalent ions currently attracts much attention (Yu, 2003; Burg et al., 2006; Okada et al., 2006; Bortner and Cidlowski, 2007; Lang et al., 2008; Hoffmann et al., 2009).

The general principles used in calculations of ion and water homeostasis in cells are known (Jakobsson, 1980; Lew and Bookchin, 1986; Novotny and Jakobsson, 1996). However, the balance of the monovalent ion fluxes in apoptotic cells has not yet been considered. The effect of alteration of distinct channels and transporters on the whole monovalent ion distribution and water balance in apoptosis was analyzed by mathematical modeling in our previous papers (Vereninov et al., 2004, 2006, 2007). The method based on step-by-step variations in distinct parameters appeared to be unsuitable and a new approach has been applied in the present study. The data obtained in our experiments with the human lymphoid cell line U937 induced to apoptosis with staurosporine were used as the experimental background (Yurinskaya et al., 2010). It was found that changes in the monovalent ion content in apoptotic cells are similar to the drift in the state of the balanced unidirectional fluxes. Therefore, the direct solution of the system of the flux balance equation was used to calculate all individual unidirectional fluxes, integral channel permeability, and other parameters that characterize specific ion pathways.

Basic equations. The movement of Na^+, K^+, and Cl^- across the cell membrane are assumed to be mediated by ion channels, the Na^+/K^+ pump and NKCC, and NC cotransporters. Net fluxes through each of these ionic pathways is distinct from zero, but all together, they can provide mass conservation, i.e., balance in the influx-efflux relationship for each species of ions associated with the specific distribution of monovalent ions between the cytoplasm and external

[1] Dedicated to the memory of L.M. Chailakhyan.

medium, which, in turn, is responsible for maintaining the cell water balance and plasma membrane potential. The following equations of the Na^+, K^+, and Cl^- influx-efflux balance were used:

$$p_{Na}u\frac{[Na^+]_i\exp(u)-[Na^+]_o}{1-\exp(u)}+J^P_{Na}+I_{NC}(1-f_{NC}) + I_{NKCC}(1-f_{NKCC}) = 0, \quad (1)$$

$$p_K u\frac{[K^+]_o\{\exp[n(u-\varphi_K)]-1\}}{1-\exp(u)} + I^P_K + I_{NKCC}(1-f_{NKCC}) = 0, \quad (2)$$

$$p_{Cl}u\frac{[Cl^-]_i-[Cl^-]_o\exp(u)}{1-\exp(u)} + I_{NC}(1-f_{NC}) + 2I_{NKCC}(1-f_{NKCC}) = 0. \quad (3)$$

The first members of these equations are net fluxes through the channels, where p_{Na}, p_K, and p_{Cl} are the channel permeability according to Goldman (Goldman, 1943; Jakobsson, 1980; Lew and Bookchin, 1986; Novotny and Jakobsson, 1996; Vereninov et al., 2004, 2007). Dimensionless potential u is related to membrane potential U in mV as follows: $U = uRT/F$. Equilibrium potential for K^+ in Eq. (2) is related to its concentration ratio as $\varphi_K = \ln([K^+]_o/[K^+]_i)$. The parameter n is introduced in the first term in Eq. (2) to account for the effect of the non-Goldman rectification in K^+ channels (Hodgkin and Keynes, 1955). If $n = 1$, the usual Goldman formula for K^+ channel flux holds. The terms J^P_{Na} and I^P_K in Eqs. (1) and (2) represent Na^+ efflux and K^+ influx through the Na^+/K^+ pump. It is accepted that $J^P_{Na} = -1.5I^P_K$. The term I_{NC} in Eqs. (1) and (3) represents influxes of Na^+ and Cl^- mediated by NC cotransport with 1 : 1 stoichiometry. The term I_{NKCC} is Na^+ influx equal to K^+ influx and half of the Cl^- influx through the NKCC cotransporter with 1 : 1 : 2 stoichiometry (Novotny and Jakobsson, 1996; Russell, 2000; Gamba, 2005). The effluxes through NC and NKCC cotransporters are $J_{NC} = f_{NC}I_{NC}$ and $J_{NKCC} = f_{NKCC}I_{NKCC}$. The coefficients f_{NC} and f_{NKCC} depend on the concentrations of Na^+, K^+, and Cl^- in the cell and medium according to the expressions

$$f_{NC} = [Na^+]_i[Cl^-]_i/([Na^+]_o[Cl^-]_o),$$

$$f_{NKCC} = [Na]_i[K]_i[Cl]_i[Cl]_i/([Na]_o[K]_o[Cl]_o[Cl]_o).$$

NC cotransport may be accomplished with a single TSC-type transporter (SLC12A3 according to the gene nomenclature) (Mount et al., 1998; Gamba, 2005) or with two functionally coupled exchangers, such as NHE (SLC9A) and Cl^-/HCO_3^- (SLC4) (Hoffmann, 1982; Russell, 2000). NKCC cotransport is performed with SLC12A1 and SLC12A2 transporters (Mount et al., 1998; Russell, 2000; Gamba, 2005).

Ion exchangers responsible for self-exchange fluxes without net flux are not included in the balance Eqs. (1)–(3). However, it should be kept in mind that the self-exchange fluxes contribute to the measured unidirectional fluxes (Hoffmann, 1982; Vereninov et al., 2007). Here, it is accepted that I_{Cl} is a measured Cl^- influx that consisted only of fluxes taken into account in balance Eqs. (1)–(3). Therefore,

$$I_{Cl} = -p_{Cl}u\frac{[Cl]_o}{1-e^u} + 2I_{NKCC} + I_{NC}. \quad (4)$$

From Eqs. (3)–(4), we have that NKCC and NC cotransport fluxes can be expressed through the chloride equilibrium potential φ_{Cl} and membrane potential u as follows:

$$I_{NKCC}(u) = I_{Cl}\frac{b}{2(1+b)}\left(\frac{1-\exp(u-\varphi_{Cl})}{1-f\exp(u-\varphi_{Cl})}\right), \quad (5)$$

where $\varphi_{Cl} = \ln([Cl]_i/[Cl]_o)$ and $f = (f_{NC} + bf_{NKCC})/(1 + b)$. Parameter b characterizes the ratio of Cl^- influxes mediated by NKCC and NC cotransporters as follows:

$$b = 2I_{NKCC}/I_{NC}. \quad (6)$$

Membrane potential u can be derived from the K^+ channel influx/efflux ratio by Ussing's formula (Ussing, 1949) or by the following more general version that accounts for the rectification in K^+ channels (Hodgkin and Keynes, 1955):

$$\frac{J^G_K}{I^G_K} = -\exp[n(u-\varphi_K)]. \quad (7)$$

The potassium influx and efflux through channels, I^G_K and J^G_K can be estimated by subtracting the pump and NKCC cotransporter fluxes from the total measured K^+ flux as follows:

$$I^G_K = \{I_K - I^P_K - I_{NKCC}\}, \quad J^G_K = \{-I_K + f_{NKCC}I_{NKCC}\}.$$

The substitution of these fluxes in Eq. (7) yields the following equation that connects membrane potential u with the total measured K^+ influx; pump K^+ influx; cotransport K^+ influx; and the ratio of Na^+, K^+, and Cl^- concentrations in the cells and medium (coefficient f_{NKCC}):

$$\{I_K - I^P_K - I_{NKCC}(u)\}\exp[n(u-\varphi_K)] - \{I_K - f_{NKCC}I_{NKCC}(u)\} = 0. \quad (8)$$

The combination of Eq. (5) with Eq. (8) gives transcendental equation for u which can be solved numerically with various b and n utilizing the data like in Table 1. Then, channel permeability and ion fluxes can be determined by formulas presented above.

Calculation of flux balance based on experimental data. Table 1 presents experimental data utilized for calculations of the partial Na^+, K^+, and Cl^- fluxes through the plasma membrane in U937 cells under normal conditions and in apoptosis caused by (1 μM)

Table 1. Experimental data on ion concentration and fluxes in normal and apoptotic U937 cells utilized for calculations of partial fluxes through various pathways and channel permeability coefficients presented in Table 2 and Fig. 1.

Cells	I_K	I_K^P	I_{Cl}	$[Na^+]_i$	$[K^+]_i$	$[Cl^-]_i$	$[Na^+]_o$	$[K^+]_o$	$[Cl^-]_o$
	$\mu mol\ g^{-1}\ min^{-1}$			mM					
Normal	4.36	3.67	32.5	39	126	41	140	5.8	116
Apoptotic	2.41	1.62	27.5	62	116	29	140	5.8	116

Note: $[Na^+]_i$, $[K^+]_i$, $[Cl^-]_i$ are intracellular ion concentrations (underscript (o) indicates extracellular concentration); I_K and I_K^P are total and pump K^+ influxes, respectively; I_{Cl} is total Cl^- influx. Data were obtained on U937-160B2 cells induced to apoptosis with 1 μM staurosporine as described (Yurinskaya et al., 2010).

staurosporine for 4 h (Yurinskaya et al., 2010). The data do not allow one to find the relationship between Cl^- fluxes through NC and NKCC cotransport pathways, i.e., parameter b, or to determine whether the rectification effect occurs in K^+-channels, i.e., parameter n. Therefore, the calculation was performed for various b and n. It should be pointed out that, according to Eq. (1), flux balance is possible only under the following conditions: $I_{NKCC} + I_{NC} \le (1.5 I_K^P)$ and $I_{NC}^{max} < (-J_{Na}^P)/(1 - f_{NC})$. The minimal value of parameter b, defined as $b_{min} = (2 I_{NKCC}^{min} / I_{NC}^{max})$, corresponds to the case when $p_{Na} = 0$. The flux balance can only occur within the range of b defined as $(2I_{NKCC}/I_{NC}) > b_{min}$. Limitations imposed by low b indicate that the simultaneous balance of K^+, Na^+, and Cl^- in cells with the properties given in Table 1 cannot be achieved without NKCC, although the value of this cotransport may be minute, as will be demonstrated below. For example, in normal cells without rectification, in K^+ channels, I_{NKCC}^{min} is less than 2% of the total potassium flux.

When the system includes only an NKCC cotransporter and rectification in K^+ channels is absent ($n = 1$), the calculated values of unidirectional partial fluxes, channel permeability coefficients, and membrane potential are as shown in Table 2. If rectification in K^+ channels takes place and $n = 3$, the K^+ fluxes through channels and NKCC cotransporter, as well as K^+ channel permeability are altered than at $n = 1$, whereas other parameters and membrane potential remain invariant. It is important that changes in channels and transporters in apoptosis deduced from the above calculations does not depend on the assumed effect of rectification in K^+ channels.

If a system includes two cotransporters, NKCC and NC, the solution of the equation set depends on the relationship between fluxes through NKCC and NC characterized by parameter b. Table 2 presents the results for the case $b = 1$. The figure shows how the computed permeability coefficients, partial fluxes, and membrane potential depend on the NKCC and NC flux ratio. It can be seen (Figs. 1a, 1b) that, when coefficient b is diminished, the channel Na^+ flux and p_{Na} decreases to zero and, furthermore, would be negative. As was mentioned above, the existence of a limit implies that the flux balance is unfeasible with an inappropriate proportion between NKCC and NC fluxes. The data obtained show that the feasible values of b in normal and apoptotic cells are distinct due to the difference in the initial cell properties given in Table 1. The limit of b also depends on rectification in K^+ channels.

The permeability of K^+ and Cl^- channels and the membrane potential estimated by calculations only slightly depend on the assumed value of parameter b. Changes in u do not exceed 2–5 mV, whereas the relative changes in p_{Cl} and p_K do not exceed 10–20 %. The calculated value of the Na^+ channel permeability depends more significantly on b, especially when the latter is low.

A change in the rectification parameter n from 1 to 3 modifies the ratio between K^+ influx and efflux through channels and, therefore, K^+ flux through NKCC cotransporter and calculated value p_K. Permeability coefficients p_{Na}, p_{Cl}, and the membrane potential are modified insignificantly under these conditions.

The calculated Cl^- influx through NKCC turns out to be small compared to the total Cl^- influx within the entire region of b values. For instance, at $b = 10$, it was only 1% of the total measured Cl^- flux (I_{Cl}) in the model without rectification and 5% with rectification. The portion of Cl^- influx through NC cotransporter in the total Cl^- influx may be more significant, especially at low b. At $b = 0.025$, I_{NC} is 40 times higher than I_{NKCC}, the absolute value of I_{NC} appears to be 5.9 $\mu mol\ g^{-1}\ min^{-1}$; it is 18% of the total Cl^- influx—32.5 $\mu mol\ g^{-1}\ min^{-1}$ (Table 1). Thus, at $I_{NC} \gg I_{NKCC}$, the partial flux I_{NC} may be identified with inhibitors.

Changes in K^+, Na^+, and Cl^- channels and transporters during the apoptosis of U937 cells caused by 1 μM staurosporine. Experimental and computed data allow one to determine the transporters and channels

Table 2. Influx (I) and efflux (J) via different pathways, permeability of different ion channels and membrane potential in normal and apoptotic U937 cells

Variables	No rectification, $n = 1$		With rectification, $n = 3$	
	normal cells	apoptotic cells	normal cells	apoptotic cells
NKCC model				
I_{NKCC}	0.148*	0.334	0.682	0.772
J_{NKCC}	−0.114	−0.179	−0.523	−0.414
J^P_{Na}	−5.51	−2.43	−5.51	−2.43
I^G_{Na}	6.06	2.55	5.92	2.32
J^G_{Na}	−0.59	−0.27	−0.57	−0.24
I^P_K	3.67	1.62	3.67	1.62
I^G_K	0.54	0.46	0.01	0.02
J^G_K	−4.25	−2.23	−3.84	−2.00
I^G_{Cl}	32.20	26.83	31.14	25.96
J^G_{Cl}	−32.27	−27.14	−31.45	−26.67
p_{Na}	0.027*	0.010	0.026	0.009
p_K	0.058*	0.042	0.001	0.002
p_{Cl}	0.484*	0.509	0.470	0.497
U	−27.4	−37.7	−27.6	−38.1
NKCC + NC model				
I_{NC}	0.287	0.645	1.361	1.538
J_{NC}	−0.028	−0.070	−0.133	−0.166
I_{NKCC}	0.143	0.323	0.681	0.769
J_{NKCC}	−0.110	−0.173	−0.522	−0.412
J^P_{Na}	−5.51	−2.43	−5.51	−2.43
I^G_{Na}	5.77	1.90	4.54	0.78
J^G_{Na}	−0.56	−0.20	−0.42	−0.08
I^P_K	3.67	1.62	3.67	1.62
I^G_K	0.55	0.47	0.01	0.02
J^G_K	−4.25	−2.24	−3.84	−2.00
I^G_{Cl}	31.93	26.21	29.78	24.42
J^G_{Cl}	−32.25	−27.08	−31.32	−26.51
p_{Na}	0.026	0.007	0.020	0.003
p_K	0.059	0.043	0.001	0.002
p_{Cl}	0.482	0.503	0.461	0.484
U	−27.6	−38.3	−28.7	−39.6

Notes: Sub- and superscripts indicate ions and pathways. NKCC and NC, cotransporters, G, channels; P, pump. I_{NKCC} corresponds to the influx of Na^+ and K^+ or to a half of the Cl^- influx mediated by NKCC cotransporter. Data are calculated by using the experimental values given in Table 1 for the model with a single NKCC cotransporter and for the model with NKCC and NC cotransporters with the equal Cl^- influxes ($I_{NKCC} = 0.5I_{NC}$). Fluxes: μmol g^{-1} min^{-1}; permeability coefficients: ml g^{-1} min^{-1}; membrane potential given in mV.

* Indicate reference values for variables presented in Fig.1 in relative units.

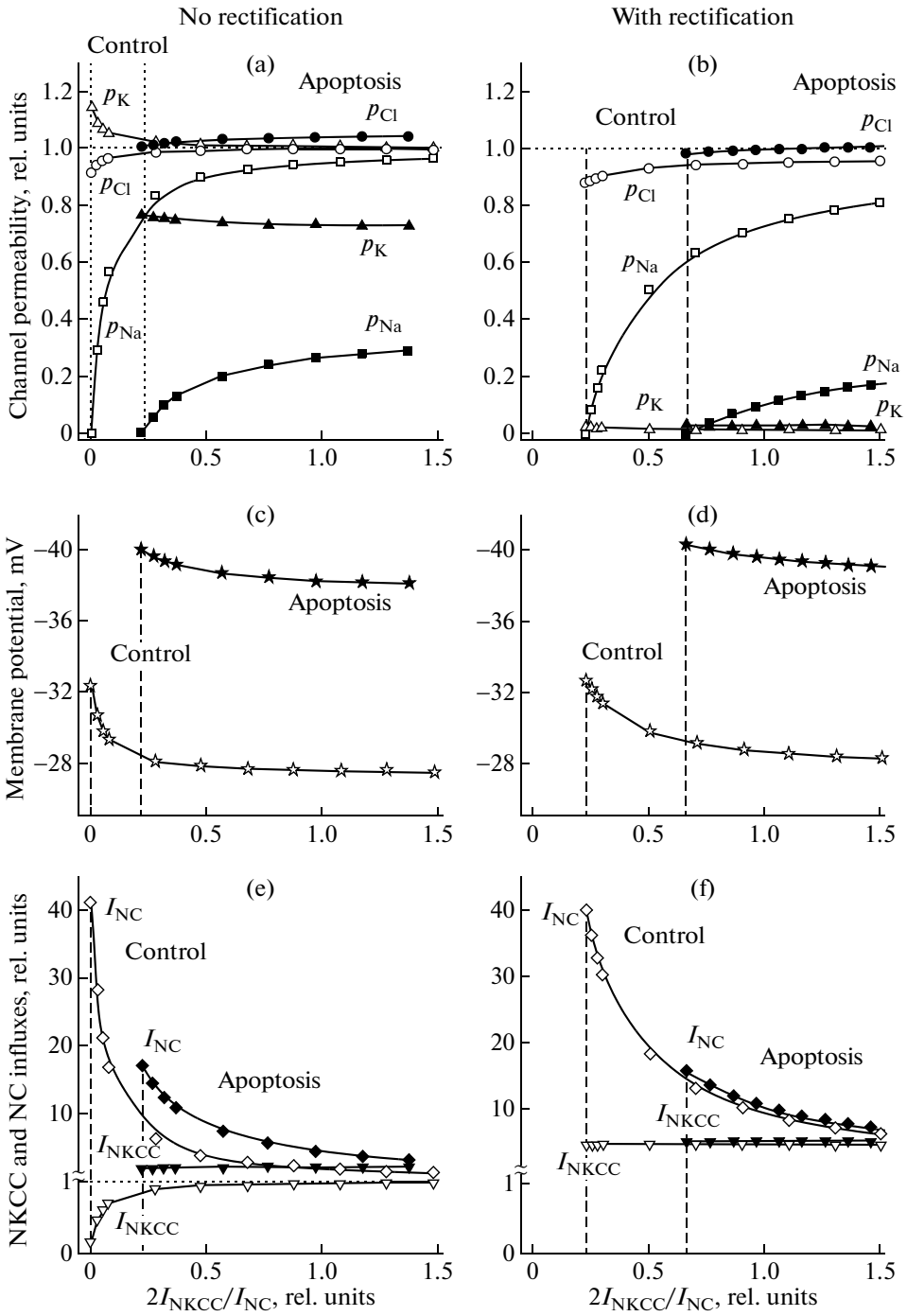

Fig. 1. Computed values of Na^+, K^+, and Cl^- channel permeability (a, b), membrane potential U (c, d) and influxes mediated by NKCC and NC cotransporters (e, f) in dependence of the ratio between Cl^- influxes through NKCC and NC. (a, c, e) Potassium channels without rectification ($n = 1$); (b, d, f) potassium channels with rectification ($n = 3$). Solid symbols, apoptotic cells; open symbols, normal cells. Variables p_{Na}, p_K, p_{Cl}, I_{NKCC} are presented in relative units with the reference values marked with asterisk in Table 2. Influx I_{NC} is given with reference to I_{NKCC} marked with asterisk in Table 2.

responsible for the modification of the ion and water balance in these apoptotic cells. One should note here that this modification is caused, not only by the changed permeability of the cell membrane, but partly by the loss of intracellular osmolytes other than monovalent ions (Yurinskaya et al., 2010). The reduced activity of the Na^+/K^+ pump is judged from the decrease in the ouabain-inhibited component of K^+ influx (I_K^P) (Vereinov et al., 2008; Yurinskaya et al., 2010). Changes in other ion pathways can only be esti-

mated by the calculated parameters. The newest and most interesting finding is a decrease in the integral permeability of Na^+ channels, p_{Na}, which occurs during apoptosis in all cells studied (Figs. 1a, 1b). Due to the decrease in p_{Na}, apoptotic cells are dehydrated, despite the reduced activity of the Na^+/K^+ pump. This conclusion holds, regardless of the assumption on the relationship between NKCC and NC fluxes and the rectification in K^+ channels. The calculation shows that the permeability of the K^+ channel is slightly diminished if rectification in K^+ channels is absent and enhanced in the model with rectification (Table 2). The permeability of Cl^- channels, p_{Cl}, remains nearly invariant in these cells. A decrease in Cl^- content in apoptotic cells is associated with the cell hyperpolarization by about 10 mV (Figs. 1c, 1d). It has been reported that apoptosis is accompanied with the cell depolarization (Bortner et al., 2001; Düssmann et al., 2003; Franco et al., 2006). This discrepancy may be due to the different cell properties or different stages of apoptosis in studies compared. However, this may also be due to the inadequacy of the mathematical model used in our study. Special analysis showed that cell depolarization can be obtained with the same experimental data if KCC cotransporter is added and it is assumed that the fluxes through KCC are decreased during apoptosis. The formula for the membrane potential U in the case of the simultaneous presence of three cotransporters in the cell membrane can be derived from Eq. (7) as follows:

$$U = \frac{RT}{F}\left\{\ln\left(\frac{[K]_o}{[K]_i}\right) + \frac{1}{n}\ln\left(\frac{I_K - I_{NKCC}f_{NKCC} - I_{KC}f_{KC}}{I_K - I_K^p - I_{NKCC} - I_{KC}}\right)\right\}, \quad (9)$$

where I_{KC} is K^+ and Cl^- influx through KCC cotransporter and f_{KC} is determined as $f_{KC} = [K]_i[Cl]_i/([K]_o[Cl]_o)$. Decreased I_{KC} in apoptosis according to the Eq. (9) results in depolarization of the cell membrane.

It was suggested that changes in NKCC and NC cotransport systems play an important role in apoptotic cell dehydration (Vereninov et al., 2004, 2006, 2007). Calculations presented here confirm that K^+, Na^+, and Cl^- flux balance in the studied cells are indeed unfeasible without at least one of these cotransporters. However, it becomes clear that fluxes through these cotransporters are rather low and could not be distinguished with inhibitors from the fluxes via other pathways. Calculations presented here show that in apoptosis these fluxes are not changed or slightly increased but whether or not the ratio between fluxes through NKCC and NC routes is altered is currently uncertain.

ACKNOWLEDGEMENTS

The work was supported by the Russian Foundation for Basic Research (project 09-04-00301a) and DFG Foundation (project 436 RUS 113/488/0-2R) and RFFI (project 06-04-04000)

REFERENCES

Bortner, C.D. and Cidlowski, J.A., Cell Shrinkage and Monovalent Cation Fluxes: Role in Apoptosis, *Arch. Biochem. Biophys.*, 2007, vol. 462, pp. 176–188.

Bortner, C.D., Gomez-Angelats, M., and Cidlowski, J.A., Plasma Membrane Depolarization without Repolarization Is an Early Molecular Event in Anti-Fas-Induced Apoptosis, *J. Biol. Chem.,* 2001, vol. 276, pp. 4304–4314.

Burg, E.D., Remillard, C.V., and Yuan, J.X.J., K^+ Channels in Apoptosis, *J. Membr. Biol.*, 2006, vol. 209, pp. 3–20.

Düssmann, H., Rehm, M., Kögel, D., and Prehn, J.H.M., Outer Mitochondrial Membrane Permeabilization during Apoptosis Triggers Caspase-Independent Mitochondrial and Caspase-Dependent Plasma Membrane Potential Depolarization: A Single-Cell Analysis, *J. Cell Sci.,* 2003, vol. 116, pp. 525–536.

Franco, R., Bortner, C.D., and Cidlowski, J.A., Potential Roles of Electrogenic Ion Transport and Plasma Membrane Depolarization in Apoptosis, *J. Membr. Biol.*, 2006, vol. 209, pp. 43–58.

Gamba, G., Molecular Physiology and Pathophysiology of Electroneutral Cation-Chloride Cotransporters, *Physiol. Rev.*, 2005, vol. 85, pp. 423–493.

Goldman, D.E., Potential, Impedance, and Rectification in Membranes, *J. Gen. Physiol.,* 1943, vol. 27, pp. 37–60.

Hodgkin, A.L. and Keynes, R.D, The Potassium Permeability of a Giant Nerve Fibre, *J. Physiol.*, 1955, vol. 128, pp. 61–88.

Hoffmann, E.K., Anion Exchange and Anion-Cation Co-Transport Systems in Mammalian Cells, *Philos. Trans. R Soc. Lond. B.*, 1982, vol. 299, pp. 519–535.

Hoffmann, E.K., Lambert, I.H., and Pedersen, S.F., Physiology of Cell Volume Regulation in Vertebrates, *Physiol. Rev.*, 2009, vol. 89, pp. 193–277.

Jakobsson, E., Interactions of Cell Volume, Membrane Potential, and Membrane Transport Parameters, *Am. J. Physiol.*, 1980, vol. 238, pp. C196–C206.

Lang, F., Gulbins, E., Szabo, I., Vereninov, A., and Huber, S.M., Ion Channels, Cell Volume, Cell Proliferation and Apoptotic Cell Death, in *Sensing with Ion Channels*, Heidelberg: Springer Verlag, 2008, pp. 69–84.

Lew, V.L. and Bookchin, R.M., Volume, pH, and Ion-Content Regulation in Human Red Cells: Analysis of Transient Behavior with an Integrated Model, *J. Membr. Biol.*, 1986, vol. 92, pp. 57–74.

Mount, D.B., Delpire, E., Gamba, G., Hall, A.E., Poch, E., Hoover, R.S., and Hebert, S.C., The Electroneutral Cation-Chloride Cotransporters, *J. Exp. Biol.,* 1998, vol. 201, pp. 2091–2102.

Novotny, J.A. and Jakobsson, E., Computational Studies of Ion–Water Flux Coupling in the Airway Epithelium. II. Role of Specific Transport Mechanisms, *Am. J. Physiol.*, 1996, vol. 270, pp. C1751–C1772.

Okada, Y., Shimizu, T., Maeno, E., Tanabe, S., Wang, X., and Takahashi, N., Volume-Sensitive Chloride Channels Involved in Apoptotic Volume Decrease and Cell Death, *J. Membr. Biol.*, 2006. vol. 209, pp. 21–29.

Russell J. M., Sodium–Potassium–Chloride Cotransport, *Physiol. Rev.*, 2000, vol. 80, pp. 211–276.

Ussing, H.H., The Distinction by Means of Tracers between Active Transport and Diffusion, *Acta Physiol. Scand.*, 1949, vol. 19, pp. 43–56.

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. 382–394.

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.

Vereninov, A.A., Yurinskaya, V.E., and Rubashkin, A.A., Apoptotic Shrinkage of Lymphoid Cells: A Model of Changes in Ion Flux Balance, *Dokl. Akad. Nauk,* 2006, vol. 411, no. 6, pp. 824–828 [*Dokl. Biochem. Biophys.* (Eng. Transl.), 2006, vol. 411, pp. 356–360].

Vereninov, A.A., Yurinskaya, V.E., and Rubashkin, A.A., The Role of Potassium, Potassium Channels, and Symporters in the Apoptotic Cell Volume Decrease: Experiment and Theory, *Dokl. Akad. Nauk*, 2004, vol. 398, no, 4, pp. 555–559 [*Dokl. Biol.* (Eng. Transl.), 2004, vol. 398, pp. 417–422].

Yu, S.P., Regulation and Critical Role of Potassium Homeostasis in Apoptosis, *Prog. Neurobiol.*, 2003, vol 70, pp. 363–386.

Yurinskaya, V.E., 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 the Mechanism of Cell Dehydration in Apoptosis, *Tsitologiya*, 2010, vol. 51, pp. 562–567 [Cell Tissue Biol. (Engl. Transl.) 2010, vol. 4, no. 5, pp. 457–463].