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CELL BIOLOGY

The Role of Potassium, Potassium Channels, and Symporters in the Apoptotic Cell Volume Decrease: Experiment and Theory[1]

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Apoptosis, programmed cell death, is a fundamental cell process, such as proliferation and senescence. Apoptosis plays an essential role in normal morphogenesis and in deletion of damaged cell, particularly those that carry mutations causing cancer and other disorders [1]. Degeneration of the neurons in many neurodegenerative diseases of humans consists in their apoptosis [2].

Ample evidences indicate an essential role of ion channels in apoptosis [3, 4]. The channels permeable for K^+ ions are of primary interest because the efflux of K^+ via channels is believed to be responsible for cell volume decrease, known as a hallmark of apoptosis. It has been shown that at least 14 species of K^+ channels are involved in apoptosis of various cells [5, 6]. The role of K^+ channels in the regulation of apoptosis is assumed to be related to the influence of intracellular K^+ concentration on apoptotic caspases and nucleases [6–8]. However, this assumption is based mostly on the study of the shift in intracellular K^+ concentration with use of a fluorescent probe and measurement of the forward light scattering. These methods are not adequate for the quantitative analysis of the ion and water balance of cells. We studied changes in the intracellular content and concentration of K^+ during apoptotic decrease in cell volume using other methods. Cell water was assayed by measuring the buoyant density of cells in a continuous Percoll gradient; cell K^+ and Na^+, by flame emission analysis. Well-known models of apoptosis were studied, namely, the apoptosis of rat thymocytes induced by dexamethasone (1 μM, 4–6 h) and the apoptosis of human lymphoma cell line U937 treated with staurosporine (1 μM, 4 h). Apoptosis was examined using confocal microscopy and flow cytometry. The methods were described in details earlier [9, 10].

Apoptosis of cells studied was found to be associated with the loss of water from thymocytes and U937 cells by 2.2 and 2.3 ml/g of protein, respectively (table). The intracellular K^+ content was decreased by 0.34 and 0.32 mmol/g, and the Na^+ content was increased by 0.13 and 0.04 mmol/g of protein. This makes it to find the proportion of ions in the total loss of intracellular osmolytes. In animal cells with a negligible internal hydrostatic pressure, the total osmolality of the intracellular media should be equal to that of external solution. Hence, in cells equilibrated with a physiological medium with an osmolyte concentration of 310 mM, the intracellular concentration should be 0.31 mmol/ml cell water. The measured apoptotic shift in water content should correspond to the total loss of osmolytes in thymocytes by 0.68 mmol/g and in U937 cells by 0.72 mmol/g of protein. The measured total decrease of intracellular cation content is equal to 0.21 and 0.28 mmol/g protein. The loss of cations must be electrically balanced by loss of anions, probably Cl^-. Thus, the total loss of the monovalent ions should be 0.42 mmol/g in thymocytes and 0.56 mmol/g protein in U937 cells, which corresponds to a decrease in the amount of cell osmolytes by 62 and 78%, respectively. Therefore, we can conclude that apoptotic loss of cell water is in fact caused mostly by the release of monovalent ions, presumably K^+. This is the first quantitative study of the ion-osmotic mechanism of apoptotic volume decrease.

The second important finding is that, in spite of the significant decrease in intracellular K^+ content per gram cell protein, its concentration in cell water decreased only slightly, by about 7–8% (table). It is unlikely that so small changes in K^+ concentration play a role in the regulation of apoptotic caspases and nucleases in living cells, as it was assumed by the authors who observed the K^+ effect on apoptotic enzymes in a cell-free system at widely varying K^+ concentrations [5, 6]. It should be mentioned that so small changes in K^+ concentration in apoptotic cells could hardly be measured with use of the K^+-sensitive fluorescent probes such as PBFI.

The question as to the role of K^+ channels in the apoptotic decrease in intracellular K^+ content is not so simple as it may seem. The ion and water balance between cell and medium is achieved when the efflux of ions via all pathways across the cell membrane is equal to their

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Table 1. Changes in K^+, Na^+, and water contents during the apoptotic volume decrease in thymocytes and U937 cells

Cells	Buoyant density of cells, ρ, g/ml	Water, ml/g protein	K^+, mmol/g protein, $x \pm S_x$	Na^+, mmol/g protein, $x \pm S_x$	$[K^+]$, mM	$[Na^+]$ mM	K^+/Na^+, $x \pm S_x$	*GIK*, %
Thymocytes, control	1.060–1.067 (5)	6.5	0.91 ± 0.04	0.18 ± 0.03	142	28	5.0 (5)	
Thymocytes, apoptosis	1.082–1.095 (5)	4.3	0.57 ± 0.10*	0.31 ± 0.04*	132	72	1.85 (5)	
U937 cells, control	1.040–1.046 (16)	10	1.1 ± 0.03	0.30 ± 0.02	111	30	3.9 ± 0.2 (35)	5
U937 cells, apoptosis	1.049–1.060 (10)	7.7	0.78 ± 0.03	0.34 ± 0.02*	102	45	2.7 ± 0.2 (25)	27

Note: Water content per gram protein, V_{prot}, was calculated as $V_{prot} = (1 - \rho/\rho_{dry})/[0.65(\rho - 1)]$, taking the density of cell dry mass, ρ_{dry}, to be 1.45 g/ml, and the proportion of protein in the dry mass to be 65% [9]. *GIK*, the "channel" portion of K^+ influx estimated by Rb^+ uptake [14], was calculated by subtracting the ouabain-sensitive "pump" portion, *PIK* (in the control, 92 ± 4% (18); in the case of apoptosis, 63 ± 3% (6)), and the bumetanide-sensitive "symport" portion, *SIK* (in the control, 3 ± 0.6 (8); in the case of apoptosis, 10 ± 2 (6)), from the total influx. *S*, standard error of the mean, the number of experiments is indicated in parentheses.
* The difference from the control value is significant at $p < 0.03$.

influx. Apoptotic cell shrinkage holds at the new balanced state is reached. Ion fluxes, intracellular ion concentrations, and cell water content depend, under these conditions, on the properties of all ion pathways across the cell membrane. The numerical solution of the system of nonlinear Eqs. (1)–(5) allows us to evaluate the changes in all variables as a function of the properties of the ion pathways across the cell membrane [11–15]. These equations are based on the macroscopic electroneutrality of the intracellular medium (Eq. (1)), the osmotic equilibrium (Eq. (2)), and the balance between fluxes of ions passing into and out of the cell (Eqs. (3)–(5)):

$$[Na] + [K] - [Cl] + zA/V = 0, \quad (1)$$

$$[Na] + [K] + [Cl] + A/V = [Na]_0 + [K]_0 + [Cl]_0, \quad (2)$$

$$p_{Na}u([Na]e^u - [Na]_0)/(1 - e^u) - \beta[Na] + S_{Na} = 0, \quad (3)$$

$$p_K u([K]e^u - [K]_0)/(1 - e^u) + (2/3)\beta[Na] + S_K = 0, \quad (4)$$

$$p_{Cl}u([Cl] - [Cl]_0e^u)/(1 - e^u) + S_{Cl} = 0, \quad (5)$$

where [Na], [K], and [Cl] are the intracellular ion concentrations, mM; $[Na]_0$, $[K]_0$, and $[Cl]_0$ are the external

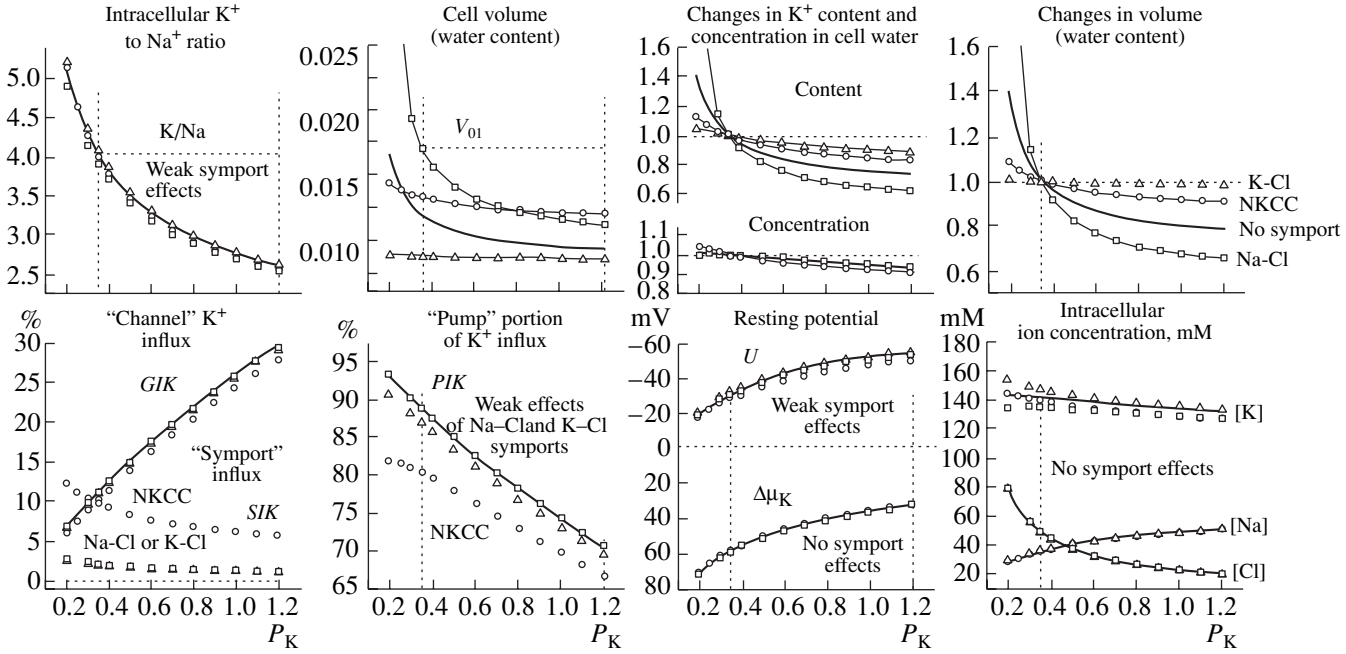

Fig. 1. Dependence of ion and water balance on K^+ channels in cells with a low resting potential and a small "channel" portion of K^+ influx. Parameters of Eqs. (1)–(5) were assumed to be the following: $z = -1.5$, $[Na]_0 = 150$; $[K]_0 = 5$; $[Cl]_0 = 155$ mM, $P_{Na} = p_{Na}/\beta = 0.15$, $P_{Cl} = p_{Cl}/\beta = 0.01$, $Q = q/28\beta$; for NKCC, $Q = 0.1$; for K–Cl and Na–Cl, $Q = 0.02$. Goldman's permeability coefficient for K^+ channels is normalized by pump coefficient, $P_K = p_K/\beta$; $\Delta\mu_K$ is the transmembrane electrochemical potential difference for K^+. V_{0l}, cell volume per unit A, ml/mmol, $V_{0l} = V/A$.

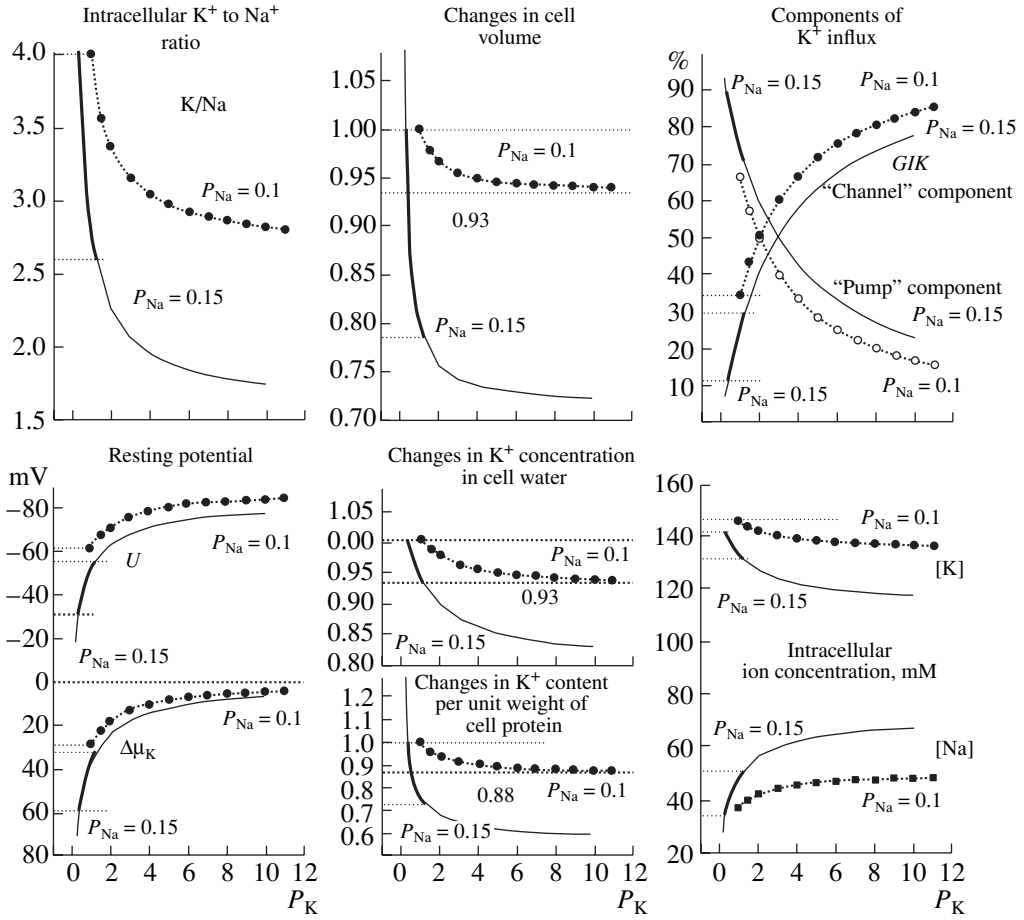

Fig. 2. Comparison of two model cells one of which decreases its volume due to the alteration of K^+ channels by about 20–25% ($P_{Na} = 0.15$, lines) and the other decreases its volume by no more than 7% ($P_{Na} = 0.1$, symbols). Calculations were the same as in Fig. 1.

ion concentrations, mM; A is the intracellular content of impermeant osmolytes, moles; z is the mean charge of the intracellular anions impermeant through the membrane (taking $z = -1.5$ equiv/mol of A), V is the cell volume (taken to be equal to water content); p_{Na}, p_K, and p_{Cl} are Goldman's permeability coefficients for ion channels; $u = UF/RT$; U is the transmembrane electrical potential difference ("resting potential"), $T = 310$ K; the second terms in Eqs. (3) and (4) are the Na^+ and K^+ fluxes via the Na, K-ATPase pump (taken to be directly proportional to [Na]) with coefficient β; S_{Na}, S_K, and S_{Cl} are the fluxes through symporters. For the NKCC symport, $S_{Na} = S_K = q\{1 - ([Na][K][Cl][Cl])/([Na]_0[K]_0[Cl]_0[Cl]_0)\}$, $S_{Cl} = 2S_K$; for the Na–Cl symport, $S_{Na} = q\{1 - ([Na][Cl])/([Na]_0[Cl]_0)\}$, $S_K = 0$, $S_{Cl} = S_{Na}$; for the K–Cl symport, $S_K = q\{1 - ([K][Cl])/([K]_0[Cl]_0)\}$, $S_{Na} = 0$, $S_{Cl} = S_K$; parameter q is the symport Na^+ or, respectively, K^+ influx. The results remain essentially the same if the relationship between pump fluxes and [Na] is assumed to be nonlinear [15].

Figure 1 shows how ion distribution, cell volume, resting potential, and ion fluxes depend on p_K in four cases: when monovalent ions can cross cell membrane only via ion channels and the Na, K-ATPase pump (solid lines); when, in addition, the coupled transport of Na^+, K^+, and two Cl^- by the NKCC symporter takes place (circles); when the K–Cl symporter operates (triangles); or when the Na–Cl symport takes place (squares), which is equivalent to the pH-coupled operation of Na/H and Cl/HCO_3 antiporters.

It can be seen that the onset or offset of symporters can change significantly K^+, Na^+ content, and cell volume (water content) even in the case of a small portion of symport (*SIK*) in the total flux (3–10%). The onset of Na–Cl symport always increases cell volume, while the onset of the K–Cl symport, conversely, decreases it. The sign of the effect of the NKCC symport depends on the initial conditions. The NKCC symport decreases the volume of cells with a high K/Na ratio (4.5), low resting potential (–20 mV), low channel and high pump portion of potassium influx, and increases it at a low K/Na ratio, high resting potential, and big channel por-

tion of potassium influx. The second important point is that the K/Na ratio, [Na^+], U, $\Delta\mu_K$, and *GIK* remain practically the same if the cell volume is changed due to the alteration of symporters. When changes in cell volume are caused by the alteration of ion channels and the Na, K-ATPase pump, they are always associated with changes in the K/Na ratio, [Na^+], U, $\Delta\mu_K$, and *GIK*. There is a marked difference between the cell shrinkage caused by the alteration of potassium channels and by the alteration of the pump or sodium channels. In the former case, it is accompanied by a decrease in the K/Na ratio; in the latter case, by its increase (data not shown). This difference may be useful for differentiating the mechanisms of cell shrinkage.

Although an increase in potassium channel permeability always leads to the loss of cell water, the degree of shrinkage strongly depends on the initial conditions, i.e., the values of the K/Na ratio, [Na+], U, $\Delta\mu_K$, *GIK*, and other characteristics. Figure 1 shows a cell with K/Na = 4 at the small channel portion of K^+ influx, *GIK* = 11%, and at the low resting potential, U = –31 mV. An increase in P_K from 0.35 to 1.2 in this cell in the absence of symport decreases the calculated cell volume by 22%, i.e., to about the same degree as in the case of the apoptosis of U937 cells (33% in thymocytes). The K/Na ratio in the model cell decreases from 4 to 2.6, while *GIK* increases from 11 to 29%. This is similar to the effects observed in living cells (table). We should not expect a complete similarity between the model and living cells because the "shrinkage" of a model cell is caused only by the redistribution of K^+, Na^+, and Cl^-, whereas the shrinkage of U937 cells and thymocytes was partly (by 22–38%) accounted for by the loss of some unknown osmolytes.

Figure 2 shows the data for a cell with P_{Na} = 0.1 instead of 0.15, which, at the same K/Na = 4, has a larger channel portion of the K influx, *GIK* = 33%, and a higher resting potential, U = –60 mV. The cell volume decrease caused by increasing P_K is limited in this case by 7%. It is accompanied by an increase in *GIK* to 70–80% and U to –80 mV. The potassium channels are not effective, because the driving force for potassium efflux, $\Delta\mu_K$, is low. Thus, although the results of the experiments with thymocytes and U937 cells showed that the apoptotic volume decrease by 62–78% was determined by the loss of ions, and computer simulation confirmed that the K^+ loss from U937 cells can be determined by the alteration of K^+ channels, the same computation indicates that these channels cause significant cell shrinkage only under limited conditions.

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