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Pump and Channel K^+ (Rb^+) Fluxes in Apoptosis of Human Lymphoid Cell Line U937

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Key Words

Rb^+ • K^+ • Pump flux • Channels • U937 cells • Apoptosis • Cell ion balance • Cell water balance • Monovalent ion flux balance

Abstract

Ouabain-sensitive (OS) and -resistant (OR) Rb^+ influx was examined in three sublines of U937 cells to compare alterations of K^+ channel permeability and the Na^+,K^+-ATPase pump leading to the shift in ion and water balance during apoptosis induced by 0.2 and 1μM staurosporine (STS) for 4-5 h. Cell K^+, Rb^+, Na^+ and Cl^- content was determined by flame photometry and ^{36}Cl distribution. Changes in cell water content were monitored by measurement of buoyant cell density and distribution of [^{3}H]-glycerol or 3-O-methyl-D-[^{3}H]glucose. Apoptosis was detected by DNA flow cytometry and light microscopy of the native cells stained with acridine orange. Treatment with 0.2 μM STS for 5 hours led to mild apoptosis with 10-13 % cell dehydration and either moderate increase of channel mediated Rb^+ influx without significant changes in the pump activity or moderate decrease of pump Rb^+ influx without significant change of channel influx, depending on the cell line used. Treatment with 1 μM STS was followed by 18-23 % cell dehydration, a decrease of the pump activity and a small or insignificant increase in the OR Rb^+ influx in all studied sublines. It is concluded that moderate apoptotic cell shrinkage may be associated with both an increase in K^+ channel permeability and inhibition of the pump whereas more remarkable shrinkage occurs presumably due to inhibition of the pump.

Introduction

Compelling evidence points to an essential role of ion channels in apoptosis [1-13]. The K^+ channels attracted attention first of all because the efflux of K^+ via channels is believed to be responsible for the volume decrease, known as a hallmark of apoptosis. It was reported that at least 14 species of K^+ channels are involved somehow in apoptosis of various cells [2]. However, calculation of the changes in ion and water balance caused by an increase in the integral permeability of K^+ channels showed that significant cell shrinkage in apoptosis due to increase in the permeability of the K^+ channels can occur only under specific conditions [14-16]. In addition, an increase in the integral permeability

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of K^+ channels conventionally associated with the opening of channels should lead to hyperpolarization of apoptotic cells whereas depolarization was mostly observed [12, 17-21]. Study of the ^{22}Na and Rb^+ fluxes in U937 cells treated with 1 μM staurosporine (STS) for 4-5 h, which was used as an established model of apoptosis with significant cell shrinkage, led us to conclude that the degradation of the sodium pump in a combination with the reduction of Na^+ influx via channels and Na^+-Cl^- cotransport, but not an increase in the permeability of K^+ channels plays the major role in alteration of the ion and water balance in apoptosis of U937 cells [15-16]. The present study reveals that an increase in ouabain-resistant (OR) Rb^+ influx, reflecting most likely K^+ channel permeability, with no pump inhibition may play a leading role in the apoptotic shift of ion and water balance of some cells but that in most cells a decrease in OS (pump) K^+ influx is more significant than the increase in OR K^+ influx.

Materials and Methods

Cell culture

Two sublines of U937 cells (U937-160B2 and U937-9957) were obtained from the Russian Cell Culture Collection (Institute of Cytology, Russian Academy of Sciences). Subline with the catalog number 160B2 is specified below as cells 1, subline with the number 9957 – as cells 2. Cells 3 correspond to the U937 cells obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). Cells in concentration of 0.7-1.0 x 10^6 cells ml^{-1} were incubated in RPMI 1640 medium (Biolot, Russia) supplemented with 10 % fetal bovine serum (FBS, HyClone Standard) at 37 °C and 5% CO_2. For induction of apoptosis the cells were exposed to staurosporine (STS, Sigma-Aldrich, Germany). A 0.2 mM stock solution of STS in DMSO was used to yield a final concentration of the drug of 0.2 - 1 μM. The final concentration of DMSO was 0.1 - 0.5 %. To study Rb^+ uptake the 50 mM stock solution of RbCl was added to 1 ml of cell suspension (≈1x10^6 cells) to yield 2.5 mM final concentration of Rb^+. Incubation of cells with Rb^+ was performed for 10 min with or without 0.1 mM ouabain (Sigma). It was shown earlier for U937 cells that Rb^+ is a good substitute for K^+ like for many other cells and that ouabain-sensitive Rb influx should be measured for short time intervals (5-10 min) because treatment of cells with ouabain for more than 10 min was followed by remarkable increase in cell Na^+ content [16]. To determine intracellular $^{36}Cl^-$ and water content cells were loaded with tracers for 1 h in RPMI medium containing ^{36}Cl (≈0.12 μCi ml^{-1}, Isotope, Russia) and a radiolabelled sugar (3-O-methyl-D-[^{3}H]glucose, about 2 μCi ml^{-1}, Amersham, U.K.) with 10 mM of carrier (Sigma) or a radiolabelled [^{3}H]-glycerol (≈ 2 μCi ml^{-1}, Amersham, U.K.) with 10 mM carrier (Reachem, Russia).

Determination of intracellular ion content

Cells were sedimented in RPMI medium and washed 5 times without resuspending by 96 mM $MgCl_2$ solution. The pellets were treated with 1 ml of 5% trichloroacetic acid (TCA) for 30 min and TCA extracts were analyzed for K^+, Na^+ and Rb^+ by emission photometry in air-propane flame (Perkin-Elmer AA 306 spectrophotometer) with solutions of KCl or NaCl (10-100 μM) and RbCl (5-10 μM) in 5% TCA as standards. The radioactivity of ^{36}Cl in TCA extracts was measured by liquid scintillation counting (Beckman LS 6500). 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 evaluated in mmol per g of protein.

Determination of cell water content

Cell water was determined by measurements of the buoyant density of cells in continuous Percoll gradient as described [22, 23]. Briefly, the density gradient was prepared from the 35-40 % Percoll solution on the base of the RPMI medium with addition of the x10 Hanks solution in proportion 1:10 to the volume of the stock Percoll (Pharmacia, Sweden). The gradient was generated in 95 mm-length 2 ml tubes at 2000 x g for 40 min (K-23 centrifuge, Janetzki, Germany). Density marker beads of 1.033, 1.049, and 1.062 g ml^{-1} (Sigma-Aldrich, Germany) were used to control the density gradient, which was about 0.005 g ml^{-1} cm^{-1}. Cell suspension (100 μl, 3-5x10^6 cells) was layered over Percoll gradient and centrifuged for 10 min at 400 x g (MPW-340 centrifuge, Poland). After isopicknic distribution the cells were taken out by a pipette, placed into 1.5 ml tubes, diluted in 4-6 volumes of the RPMI medium and sedimented for 5 min at 300 x g (MPW-310 centrifuge, Poland). The pellets were assayed for ions and protein as described. The water content per g protein, $v_{prot.}$, was calculated as $v_{prot.} = (1-\rho/\rho_{dry})/[0.79(\rho-1)]$, where ρ is the measured cell buoyant density and ρ_{dry} is the density of cell dry mass. The latter was taken as 1.35 g ml^{-1}, while the share of the protein in dry mass as 0.79.

For determination of cell water content by radiolabelled glucose and glycerol the cells were processed as with ^{36}Cl. The buoyant density of cells is a much more sensitive and reliable method for determination of cell water than the technique using intra- and extra-cellular water markers. The difference in the density by 0.005g ml^{-1}, which corresponds to the shift in cell water content by about 10 %, was associated in our experiments with the displacement of cells in the gradient tubes by approximately 1cm. Dispersion of the data obtained by radiolabels was much higher than that found by the measurement of buoyant density.

DNA flow cytometry

For determination of the G0/G1, S, G2 cells and cell fragments with the hypodiploid DNA content about ≈5x10^5 cells were treated with 0.5 ml solution containing 0.02% ethylenediaminetetracetic acid (EDTA), 15 mM $MgCl_2$, 0.1% Triton X-100, 20 μg/ml Ethidium bromide (EB), and 40 μg/ml olivomycin, pH=7.4 for 20-24 h at 4-6°C. Fluorescence was measured at > 600 nm and excitation within 380-470 nm by using a flow cytometer described earlier [24].

Cells	Sample	Experiments with 0.2 μM staurosporine			Experiments with 1 μM staurosporine		
		Pump	Channels	Pump/Channels	Pump	Channels	Pump/Channels
1	Control	14.1 ± 0.6 (28) [a]	2.7 ± 0.1 (28)	5.3 ± 0.3 (28)	13.6 ± 0.8 (21)	4.1 ± 0.2 (21) [c]	3.5 ± 0.2 (21)
	Apoptosis	14.3 ± 0.8 (28)	4.4 ± 0.3 (28)	3.4 ± 0.1 (28)	6.0 ± 0.6 (21)	4.7 ± 0.2 (21) [d]	1.3 ± 0.1 (21)
2	Control	12.0 ± 0.5 (24) [b]	6.9 ± 0.3 (24)	1.8 ± 0.1 (24)			
	Apoptosis	7.5 ± 0.6 (24)	6.9 ± 0.5 (24)	1.2 ± 0.1 (24)			
3	Control	17.4 ± 1.1 (9)	3.4 ± 0.2 (9)	5.3 ± 0.4 (9)	17.4 ± 1.1 (9)	3.4 ± 0.2 (9)	5.3 ± 0.4 (9)
	Apoptosis	13.4 ± 0.8 (9)	4.5 ± 0.4 (9)	3.2 ± 0.4 (9)	5.2 ± 0.6 (9)	3.4 ± 0.2 (9)	1.6 ± 0.2 (9)

Table 1. Effect of apoptosis induced by 0.2 and 1 μM staurosporine on Rb^+ influx via the pump and channels in different sublines of U937 cells. Rb^+ influx is given as the Rb^+ uptake for 10 min in the medium with 2.5 mM Rb^+, μmol per g of cell protein. The influx via channels or pump is defined as uninhibited or inhibited by 0.1 mM ouabain component. Cells 1 - U937-160B2, Cells 2 - U937- N9957, Cells 3 - U937-DSZM. Cells were incubated with staurosporine for 4-5 h. Means ± se, number of experiments is given in parentheses. Significant differences between means at $P < 0.05$ when they are non-evident are marked by superscripts a, b and c, d.

Table 2. Separation of K^+ pathways by the effects of 0.1mM ouabain and 0.05 mM bumetanide on the 10 min Rb^+ uptake in U937-160B2 cells incubated with and without 1μM STS for 4 h. Rb^+ uptake is given in μmol per g of cell protein. Significant differences between means when they are non-evident are marked by superscripts a, b. One and the same number of measured samples denoted in brackets indicates the paired comparison of results obtained in the same experiment. For other details see legend to Table 1.

Pathways	Control	STS
All	18.2 ± 1.2 (16)	11.3 ± 0.8 (16)
Ouabain-Res	4.0 ± 0.3 (16)	4.6 ± 0.3 (16)
Ouabain-Sens	14.2 ± 1.0 (16)	6.7 ± 0.8 (16)
All	24.7 ± 1.3 (22) [a]	16.6 ± 1.6 (8)
Bumetanide-Res	21.3 ± 1.1 (22) [b]	15.1 ± 2.1 (8)
Bumetanide-Sens	3.4 ± 1.0 (22)	1.4 ± 1.3 (8)
All	17.9 ± 0.9 (6)	
Ouabain-Res	3.9 ± 0.2 (6)	
(Ouab. + Bum)-Res	2.4 ± 0.2 (6)	
Bumetanide-Sens	1.6 ± 0.3 (6)	

A minimum of 10,000 particles were recorded for each histogram.

Microscopy

Cell preparations were stained for 5 min by acridine orange (AO, 2 μg/ml) in a combination with EB (20 μg/ml). The fluorescence was excited with an argon laser at 488 nm and passed through the 500-550 nm spectral window for the "green" AO fluorescence and 600-700 nm window for the "red" AO and EB fluorescence. LSM 5 Pascal microscope (Carl Zeiss, Germany) was used. The images were combined with a Zeiss LSM image browser.

Statistics

Data were analyzed by Student's *t* test and considered significantly different at $p<0.05$.

Results

Inhibition of the Na^+,K^+-ATPase pump by ouabain decreased Rb^+ influx in cells 1 and 3 on average by 84 % and in cells 2 by 63 % (Table 1). The residual component of the Rb^+ influx (ouabain-resistant, OR) is presumably mediated by K^+ channels, because the effect of bumetanide and DIOA, used as inhibitors for other possible K^+ pathways (NKCC, KCC) was found to be small (Tables 2, 3).

Essential difference in the changes of the ouabain-sensitive (OS) Rb^+ influx and OR Rb^+ influx in apoptosis were found in the studied sublines of U937 cells (Table 1). Treatment of cells 2 with 0.2 μM staurosporine (STS) for 4-5 h was followed by a 1.6-fold decrease in the pump Rb^+ influx without changes of the influx via channels. A similar decrease in the pump Rb^+ influx was found earlier in the subline U937-160B2 (Cells 1) following treatment with 1 μM STS [16]. In contrast, no changes in the pump Rb^+ influx and 1.6-fold increase in the Rb^+ influx via channels were observed in cells 1 following treatment with 0.2 μM STS. Treatment of cells 3 with 0.2 μM STS was associated with both, a moderate decrease in the pump and a moderate increase in the OR Rb^+ influx. Accordingly, the response of cells 3 was intermediate.

Drugs	Rb^+ uptake	K^+/Na^+	K+Na+Rb	k, 10^{-3} min^{-1}
None	62.3 ± 1.7 (8)	4.7 ± 0.1 (8)	1.32 ± 0.03 (8)	6.3 ± 0.7 (12)
DIOA, 0.5 h	53.8 ± 2.9 (8)	3.4 ± 0.1 (8)	1.23 ± 0.02 (8)	4.9 ± 0.7 (12)
None	57.8 ± 2.7 (12)	4.1 ± 0.3 (12)	1.37 ± 0.03 (12)	5.8 ± 0.7 (10)
DIOA, 4 h	46.0 ± 2.2 (12)	3.3 ± 0.1 (12)	1.23 ± 0.03 (12)	4.9 ± 0.6 (10)
None	54.3 ± 2.8 (12)	4.5 ± 0.2 (12)	1.32 ± 0.03 (12)	6.1 ± 0.6 (14)
Bumetanide 0.5 h	50.5± 2.7[a] (12)	4.6 ± 0.2[c](12)	1.29 ± 0.03 (12)	6.0 ± 0.7 (14)
None	55.8 ± 2.8 (8)	4.8 ± 0.2 (8)	1.34 ± 0.04 (8)	6.8 ± 0.9 (6)
Bumetanide, 4 h	49.6 ± 1.9[b] (8)	3.8 ± 0.1 (8)	1.21 ± 0.05 (8)	8.0 ± 0.3 (6)

Table 3. Effects of 0.1 mM DIOA and 0.05 mM bumetanide on 0.5 h Rb^+ uptake, K^+/Na^+ ratio, cation content and Rb^+ efflux rate constant (k) in U937-160B2 cells. Rb^+ uptake is given in μmol per g of cell protein, cation content in mmol per g of cell protein. Drugs were applied for 0.5 or 4 h, as indicated. For determination of Rb^+ efflux rate constant the cells were incubated in RPMI media with 1 mM RbCl for 24 h and then transferred into Rb free medium for 30 min. k was calculated by the formula: $k = - 1/t \ln(y_t/y_o)$, where y_t/y_o is a Rb^+/K^+ ratio in cells to the initial one. The drug effects on Rb^+ uptake, K^+/Na^+ ratio and cation content are significant at $P < 0.05$ in all cases except marked by superscripts a, b, c, where P = 0.059, 0.054 and 0.059, respectively. Differences between k values obtained with and without drugs are not significant. For other details see legend to Table 1.

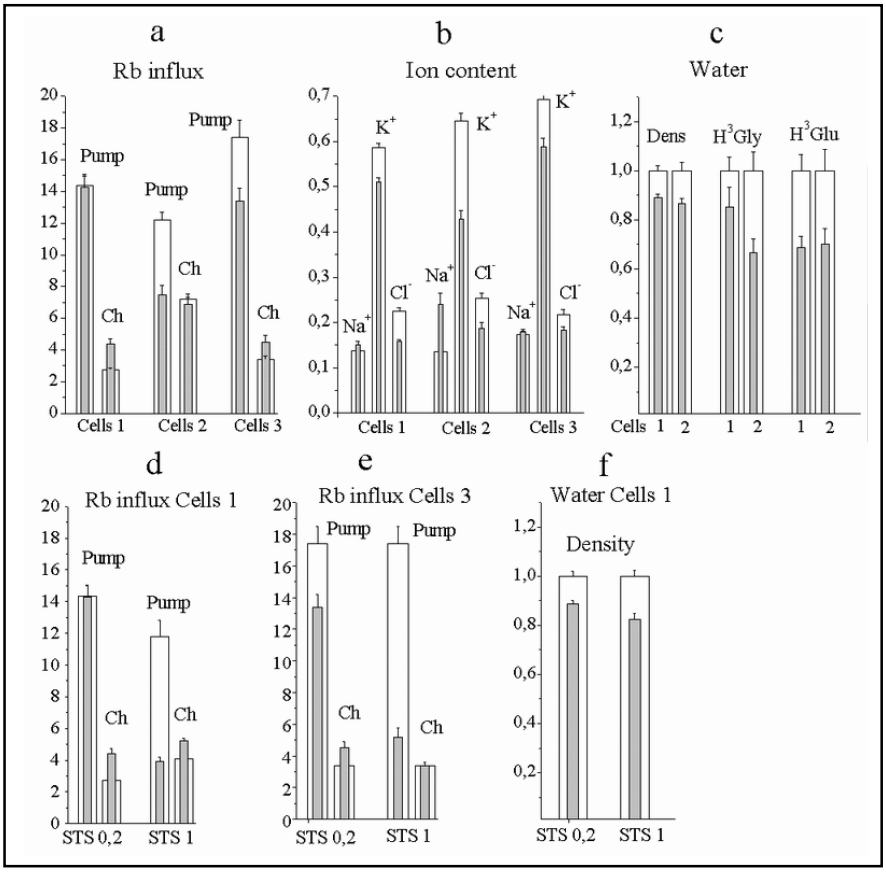

Fig. 1. Rb^+ influx (a, d, e), intracellular K^+, Na^+, Cl^- content (b) and changes in water content (c, f) in three sublines of U937 cells untreated (open columns) and treated (filled columns) with 0.2 (a, b, c) or 1 μM staurosporine (as indicated) for 4-5 h. Vertical axis: a, d, e - Rb^+ influx estimated as uptake for 10 min, μmol g^{-1}, P - pump component, Ch - channel component; b - ion content, mmol g^{-1}; c - water content in relative units, determined by buoyant density (Dens) or by radiolabelled 3-O-methyl-D-[^{3}H]glucose (^{3}HGlu) or [^{3}H]-glycerol (^{3}HGly). Cell sublines and STS concentration are indicated on the horizontal axis. Cells 1 - U937-160B2, Cells 2 – U937- N9957, Cells 3 - U937-DSZM. Values are means ± se for 8-32 determinations.

All studied sublines responded to treatment with 1 μM STS with a significant decrease in the pump Rb^+ influx and with moderate or insignificant change in the OR influx.

Generally, the studied sublines seem to be similar in the K^+, Na^+ and Cl^- content under normal conditions (Fig. 1). Apoptosis of all cells treated with 0.2 μM STS

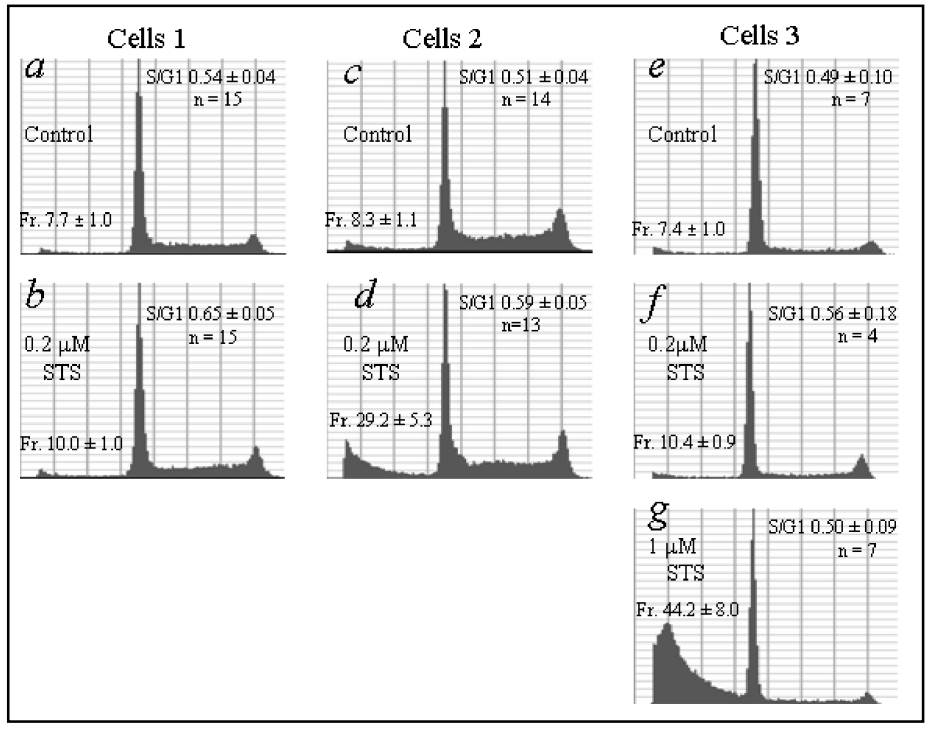

Fig. 2. DNA histograms of U937 cells untreated (a, c, e) and treated (b, d, f, g) with staurosporine in indicated concentration. Cells 1 - U937-160B2, Cells 2 – U937- N9957, Cells 3 - U937-DSZM. The histograms represent typical experiments. Staurosporine was applied for 5 h (b, d, f) or 4 h (g). The mean values of S/G1 ratio and fragment numbers (Fr.) for n experiments are given on the plots. Vertical axis - cell number. Horizontal axis - DNA content.

was accompanied by a decrease in K^+ and Cl^- content and by a decrease in the intracellular K^+/Na^+ ratio. The more remarkable increase in Na^+ content in Cells 2 correlated with more strong alteration of the pump. Inhibition of the pump in cells 3 was somewhat less and no significant increase in Na^+ occurred. The most essential initial difference between cells 1 and 2 was in the relationship between the pump and the channel component of the $Rb^+(K^+)$ fluxes. The pump influx was higher than the OR flux component in cells 1 and 3 by a factor 5.3 vs. 1.8 in cells 2 (Table1).

The differences in the changes of OR and OS Rb^+ influxes in cells 1 treated with 0.2 and 1 μM STS correlated well with cell dehydration (Fig. 1, *f*). In the experiments presented in Fig.1 the water content of cells 1 approached in mild apoptosis (0.2 μM STS) 0.89 ± 0.01 of normal cells ($n = 8$, $p < 0.001$) and in deeper apoptosis (1 μM STS) 0.82 ± 0.02 of normal cells ($n = 4$, $p < 0.005$). In another series of experiments the apoptosis of cells 1 treated with 1 μM STS was associated with cell dehydration down to a value 0.77 [23]. Relative changes in water content in the studied cells found by different methods are shown in Fig. 1, *c*. Our experience has shown that among all the methods used the measurement of the buoyant cell density provides the most reliable data at least when small relative changes in cell water content are to be detected, as in the study of mild apoptosis. The calculation of absolute value of cell water content found by buoyant density measurements depends (almost linearly) on the assumed density of dry cell mass whereas calculation of the relative changes practically does not. Dispersion of the data obtained by using radiolabelled glycerol or 3-O-methyl-D-glucose was much higher than that for the data obtained by buoyant density. The apparent difference in the relative changes in cell water content obtained by different methods for Cells 1 and 2 in Fig. 1, *c* is not significant.

Substantial differences between the cell lines in the alterations of OS and OR fluxes were observed only in the case of mild apoptosis. Accordingly, the degree of apoptosis was important. Therefore, the DNA flow cytometry of fixed cells and cell fragments stained with EB and light microscopy of unfixed cells stained with AO were performed to compare the apoptosis induced by 0.2 μM STS in the studied sublines.

Typical DNA histograms obtained by flow cytometry are presented in Fig. 2. The studied sublines did not differ in the rate of proliferation and, hence, in the initial DNA histograms. The ratios S/G1 were 0.54 ± 0.04 ($n = 15$), 0.51 ± 0.04 ($n = 14$) and 0.49 ± 0.1 ($n = 7$) in the control cells 1, 2 and 3, respectively. The histogram for cells 1 treated with 0.2 μM STS did not differ from that for the control cells whereas cells 2 treated with 0.2 μM STS as well as all cells treated with 1 μM STS show significant increase in the number of cell fragments. Differences in histograms for cells 1 and 2 treated with 0.2 μM STS

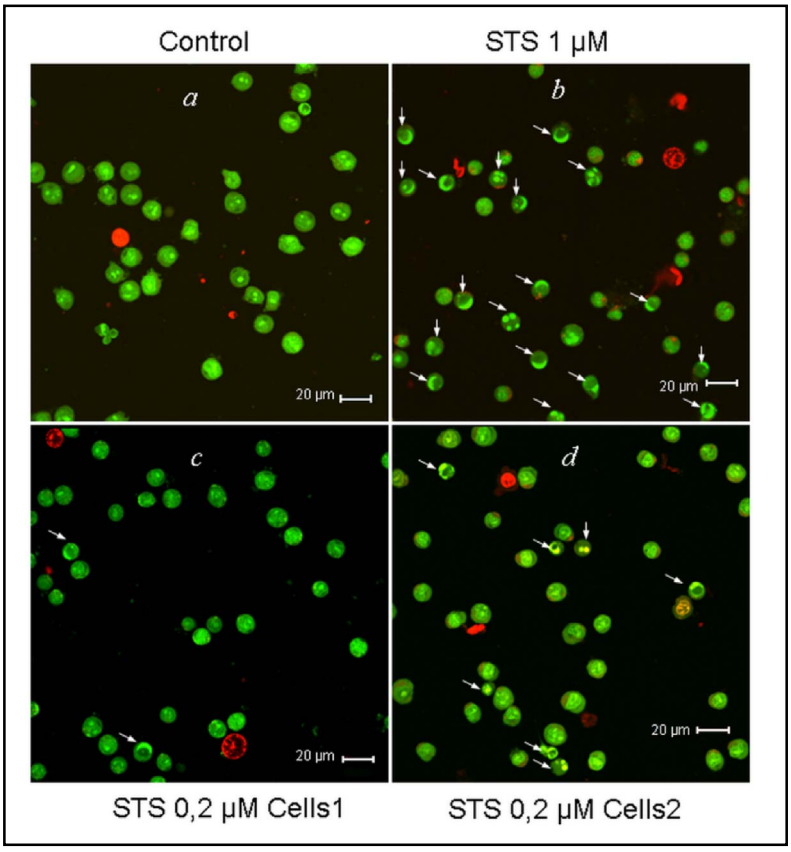

Fig. 3. Confocal images of U937 cells incubated with STS (b, c, d) or without inductors of apoptosis (a). Cells were stained by AO and EB. b - STS 1 μM, 4.5 h, Cells 3; c - STS 0.2 μM, 5 h, Cells 1; d - STS 0.2 μM, 5 h, Cells 2. Apoptotic cells with condensed chromatin are shown by arrows. Red cells are permeable for EB.

indicate that cells 2 underwent more profound apoptosis. The percentage of cells in G1 was slightly decreased in all cell types undergoing mild apoptosis.

Vital staining of U937 cells with AO discloses apoptosis by specific chromatin rearrangement much earlier than cells become permeable for EB being colored intensively red. Alterations typical for apoptosis were observed in all studied sublines when 1 μM STS was used (Fig, 3, *b*). However, only in a small portion of cells 1 and 2 apoptotic changes were observed after treatment with 0.2 μM STS (Fig. 3, c, *d*). It is remarkable that the number of apoptotic cells in the first case was less than in the latter. This proportion corresponds to the difference in the changes of Rb^+ fluxes in cells 1 and 2. Evidently, inhibition of the pump is associated with more pronounced apoptosis.

Discussion

The present data showed that two different ionic mechanisms may cause the shift of ion and water balance in the mild apoptosis of the studied sublines of U937 cells in spite of the general similarity in the redistribution of Na^+, K^+ and Cl^- and cell shrinkage. Apoptotic inhibition of the sodium pump is the most conspicuous effect in one subline while the increase in ouabain-resistant Rb^+ flux presumably reflecting K^+ channel activity in another subline. Both effects were observed at the stage of apoptosis when alterations of cell morphology can be revealed by microscopy only in single cells and the number of fragments in the DNA cytogram obtained by flow cytometry increased only slightly. It is a wide spread opinion that ionic changes precede several other hallmarks of apoptosis such as DNA laddering, activation of caspases, and chromatin condensation [12, 25-29]. Our data show that changes in Rb^+ fluxes are the most remarkable feature in early stages of apoptosis.

The idea that K^+ channels play a central role in ionic regulation of apoptosis is very popular [2, 4, 6-10, 12] However, most of the arguments are indirect and may relate to quite different roles of K^+ in apoptosis. First, K^+ channels are very important for maintaining the potential difference across the plasma membrane and thus on membrane potential sensitive cellular signaling. Second, cell shrinkage is believed to be a prerequisite of apoptosis [30]. As K^+ ions are the dominant ionic species inside the cell their impact on water balance should be major. Indeed, the loss of cellular K^+ during apoptosis is well established. However, the fact that K^+ exits via channels

does not necessarily mean that opening of channels or increase in their number is the prime cause of the loss of K^+. Alteration of ion and water content in the time scale of hours in apoptosis of cells with a rate of ion exchange of order 0.1-0.01 min^{-1}, e.g. in proliferating U937 cells, should be considered as a slow drift of a balanced state [14-16]. It is clear that the balanced state depends on the properties of all ionic pathways through the plasma membrane as well as on the amount of the charged and uncharged intracellular osmolytes which do apparently not permeate through the plasma membrane of an unchallenged cell.

Computer modeling of the balance of major ion fluxes in cells with the Na^+,K^+-ATPase pump, electroconductive K^+, Na^+, Cl^- channels, and NKCC, KCC, and Na-Cl cotransport show that the effect of K^+ channel opening on the ion distribution and cell water balance depends highly on the initial state of the system. In particular, it should be small in high potassium cells with a relatively high resting potential because the transmembrane electrochemical potential difference for K^+ is in this case small. The conditions determining the shift in K^+, Na^+ and water content following K^+ channel opening in apoptosis of U937 cells treated with 1 μM STS, were considered earlier [14-16]. The present data demonstrate clearly that the mild apoptosis with 10-13 % cell shrinkage in one subline of U937 cells is associated with the increase in Rb^+ (K^+) influx via channels and no significant alteration of the pump flux whereas in other cell sublines a similar apoptosis is accompanied by pump inhibition with no or moderate alteration of K^+ channel flux. It is important to note, however, that the data presented do not allow the conclusion that the opening of K^+ channels or the pump inhibition described is an ultimate cause of the apoptotic shift of ion and water balance in the studied U937 cells. Our computer modeling of the flux balance of all major monovalent ions Na^+, K^+, and Cl^- (unpublished) shows that properties of pathways other than the Na^+,K^+-ATPase pump and K^+ channels are altered in apoptosis of the studied U937 cells. Further analysis will be required to define those pathways.

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