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THE STUDY OF Ca^{2+} INFLUX IN HUMAN ERYTHROCYTES IN ISOTONIC POLYETHYLENE (GLYCOL) 1500 (PEG-1500) AND SUCROSE MEDIA

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Effects of isotonic solutions of polyethylene (glycol) 1500 (PEG-1500) and sucrose on Ca^{2+} influx into ATP-depleted red blood cells were studied using the Ca^{2+}-sensitive fluorescent dye fura-2AM. When incubated in isotonic low ionic strength media (containing 2 mM $CaCl_2$ in addition to sucrose and PEG-1500), the initial rate of Ca^{2+} influx was higher than that for the cells in physiological (normal ionic strength) medium. After 20 minutes of incubation in the PEG-1500-containing solution, a 10-fold increase of Ca^{2+} influx was observed, whereas in the sucrose medium the rate of Ca^{2+} influx decreased compared to that in physiological medium. ^{1}H-NMR data provided no evidence of direct interaction between PEG-1500 and the erythrocyte membrane. Moreover, PEG-1500 did not affect lipid peroxidation (LPO) induction in erythrocyte membranes. We propose that a change in the hydrogen environment of Ca^{2+}-ATPase of the erythrocytes suspended in the PEG-1500 solution is the primary cause of altered Ca^{2+} homeostasis in these cells. The activation of the Ca^{2+}-ATP-ase in sucrose medium may result in an incomplete suppression of the Ca^{2+}-pump activity in ATP-depleted cells, which is accelerated when calmodulin binds with the Ca^{2+}-ATP-ase under the conditions of rapid Ca^{2+} accumulation.

Key words: ATP-depleted erythrocytes, calcium influx, sucrose, polyethylene (glycol), fura-2.

Osmotically active compounds, such as sucrose and low molecular weight polyethylene (glycol), are widely used for cryopreservation of different cells and tissues [1]. Sucrose per se is a sufficient cryoprotective for erythrocytes [2]. As for PEG-1500, it was recently shown [3] that this substance is highly effective at protecting human red blood cells from damage during freezing-thawing manipulations. However the action mechanism of these substances remains unknown, and the data in literature are controversial [4, 5]. It has been postulated that sucrose and PEG-1500 have the same effect on the activity of acetylcholinesterase enzyme, vesiculation and rapid hemolysis of human erythrocytes as the anion detergent SDS [5]. There was also no difference between the effect of sucrose and PEG-containing media on the $Na^+/K^+/H^+$ exchanger in the human red blood cells (RBCs) (unpublished data of I. Bernhardt et al.).

Nevertheless it is known that sucrose is added to the preserving solution primarily to keep the osmotic balance between the cells and extracellular media [1, 2]. At the same time PEG demonstrates cryoprotective properties even in the absence of other additives [1, 3]. At high concentration, however, PEG causes cells fusion [6–8], which is not apparent in sucrose media [9].

In this study, we have investigated the effects of sucrose and PEG on intracellular Ca^{2+}. Calcium homeostasis is an important property of red blood cells, depending predominantly on the balance between a low passive Ca^{2+} permeability and a highly active plasma membrane Ca^{2+} pump. Free cytosolic Ca^{2+} concentration is maintained at a low level, about 100 nM. Many factors alter the function of the Ca^{2+} pump and hence free $[Ca^{2+}]$. These may act by influencing protein composition, location and redistribution of the lipids within the membrane. For example, this is observed during the activation of LPO [10] and in certain pathological diseases (such as malaria, thalassemia, sick cell anemia etc.) [11–13]. In addition, ageing of erythrocytes leads to the sharp increase of the cell membrane permeability for calcium, as postulated in [14, 15]. Ca^{2+}-ATPase activity is depressed by some detergents (Triton X-100, Twin 80) at low concentrations, whilst both Ca^{2+}-ATPase and Na^+,K^+-ATPase are inhibited by alcohol, glycerol and PEG [16–20]. However, the conditions used may distort the structure and composition of the

membranes [19—21], thus complicating the interpretation of these findings.

Here, we have used cell permeable fluorescent dye (fura-2AM) to study the effect of the isotonic solutions of sucrose and PEG-1500 on calcium influxes in ATP-depleted RBCs.

Materials and Methods

Reagents. Inorganic salts, sucrose and glucose were of analytical grade. Inosine and iodoacetate were obtained from «Sigma» (USA), Tris and EGTA from «Fluka» (Switzerland), MES from «SERVA» (Germany), PEG-1500 from «Merck/Sigma» (Germany) and fura-2/AM from «Molecular Probes» (Netherlands).

Blood and solutions. Fresh human RBCs were collected from healthy donors and washed three times (1200 g, 5 min) at a room temperature in the high ionic strength (HIS) solution containing (mM): NaCl 145, glucose 10, morpholinoethanesulphonic acid/Tris-(hydroxymethyl)aminomethane (MES/Tris) 10, pH 7.4. Supernatant and buffy coat were removed by aspiration. Experiments were carried out in the HIS solution or in solutions where 145 mM NaCl was replaced by 200 mM sucrose (LIS) or 76 mM PEG-1500 (PEG-1500). In addition, all solutions contained 2 mM $CaCl_2$. The osmolarity of the solutions was determined using an osmometer (Knauer, Germany) and found to be 295 ± 5 mOsm for HIS and 255 ± 5 mOsm for LIS and PEG solutions. Osmolarity of the LIS and PEG solutions was slightly reduced in comparison with the physiological one (HIS) to avoid an initial shrinkage of the cells, which is observed when 250 mM sucrose used.

Measurement of the Ca^{2+} influx of ATP-depleted RBCs. Ca^{2+} uptake was measured in ATP-depleted RBCs that had been pre-treated by the method described in [22, 23]. RBCs at 1% hematocrit were incubated at 37 °C in a medium containing (mM) NaCl – 140, KCl – 5, iodoacetate – 6, inosine – 6, pH 7.4 (medium A). After at least 2 hours from the beginning of the incubation 0.5 μM fura-2 (taken from a 5 mM stock solution, in DMSO) was added to the medium (45 min loading procedure). The cells were then washed three times (15 s, 12,000 g) with the medium A to eliminate the excess of extracellular fura-2, then diluted (also in medium A) to an hematocrit ~40% and stored for 15÷30 min. Aliquots of the RBCs suspension were diluted to ~0.067% hematocrit in the Ca^{2+}-containing solution studied and transferred into a quartz cuvette for fluorescence measurements (spectrophotometer FluoroMax-2, Jobin Yvon, France). Ca^{2+} influx was calculated from the changes of $[Ca^{2+}]_i$ and expressed in μmol×L cells^{-1}×h^{-1}.

Intracellular free Ca^{2+} levels were determined using a two-wavelength method as described in [22, 23]. $[Ca^{2+}]_i$ was determined from the standard equation: $[Ca^{2+}]_i = K_D \times Q \times (R\text{-}R_{min})/(R_{max}\text{-}R)$, where K_D is the Ca^{2+} dissociation constant of fura-2; R represents the ratio of the fluorescence intensities measured at 340 nm and 380 nm; R_{max} and R_{min} were found when fura-2 was saturated with Ca^{2+} and when completely free of Ca^{2+}, respectively. Q is the ratio of the minimum/maximum fluorescence intensity at 380 nm, i.e. the fluorescence intensity measured when fura-2 is free of Ca^{2+}, and saturated with Ca^{2+}, respectively. R_{min} and R_{max} were estimated under all experimental conditions separately (but see below). To determine R_{max} 0.01% Triton was added to the cuvette with the fura-2 loaded RBCs. At the end of the experiment 10—20 mM EGTA was added to estimate R_{min}.

K_D values were determined after fura-2 loaded RBCs were lysed with Triton X-100 in HIS solution. The dissociation constant of Ca^{2+} for fura-2 measured under our experimental condition was 155 nM. This value is in good agreement with the K_D = 140 nM estimated by Molecular Probes in MOPS buffer, pH 7.2 at 22 °C in the absence of Mg^{2+}.

Quenching of the fura-2 fluorescence ratio in LIS and PEG media was observed after lysis of the RBCs [23]. Therefore, the values for R_{max} and R_{min} obtained in the experiments with RBCs in HIS solution were used for the experiments in LIS and PEG solutions.

LPO analysis in the RBCs treated with PEG-1500. Thiobarbituric acid (TBA) test was used for LPO analysis in the erythrocytes incubated with PEG-1500. Briefly equal volumes (0.1 ml) of PEG-1500 (HIS in the control samples) solution and washed packed cells (80% hematocrit) were mixed and incubated for 45 min. The reaction was stopped by addition of 0.1 ml of trichloroacetic acid (TCA) (100%). Then 1.7 ml of the HIS solution, 2 ml of 30% TCA, 0.2 ml 5n HCl and 2 ml of 0.75% TBA were added. The mixtures were heated on the water bath at 100 °C for 1 h, cooled and centrifuged for 10 min at 1200 g. Concentration of malonodialdehyde (MDA) was found as the absorption data of the supernatants measured at 535 nm (PYE UNICAM SP 8000, England).

PEG-1500 binding studies using ^{1}H-NMR spectroscopy. To study PEG-1500 binding with erythrocyte membrane we used ^{1}H-NMR spectroscopy. It is known that 1H bands from CH-, CH_2-, CH_3-groups of absorbed polymer are wide and are not registered in ^{1}H-NMR spectra of the dense cell suspension but only in the lysate obtained after the cells lysis with Triton X-100.

The experiments were performed with NMR spectrometer (TESLA BS 567A, Czechoslovakia) operated at 100 MHz for ^{1}H.

Bromthymol blue (BTB) binding assay. BTB binding to erythrocytes suspended in physiological and PEG-1500 media was studied as described in [24]. Concentration of erythrocyte membrane protein was $40 \div 50 \cdot 10^{-6}$ g. Sorption parameter for BTB binding (amount of binding sites, n) was calculated from the Scatchard's plots.

Hematocrit measurements. Hematocrit (Ht, %) of the cell suspensions used in the study was determined by microhematocrit or cyanmethemoglobin methods, in common usage.

Statistical treatment of the results. Values are given as the result of at least 3 independent experiments carried out on blood of different donors ± SD.

Results

The rates of calcium influx, calculated from the records of time-dependent changes of intracellular free calcium concentration for the ATP-depleted cells incubated in HIS, LIS and PEG-1500 solutions, are shown in Table.

The rate of calcium influx for the cells transferred from the ATP-depleted medium into HIS solution was 22.78 ± 7.61 $\mu M \times L\ cells^{-1} \times h^{-1}$ over the first 10 min of incubation, and then slightly decreased to 19.31 ± 3.39 $\mu M \times L\ cells^{-1} \times h^{-1}$ during the next 35 min of incubation.

The cells transferred into LIS solution demonstrated increased rate of calcium uptake over the initial 10–15 min. period than that in HIS solution (27.58 ± 8.96 $\mu M \times L\ cells^{-1} \times h^{-1}$). However the rate significantly decreased to 9.13 ± 1.51 $\mu M \times L\ cells^{-1} \times h^{-1}$ during the last 35 min of incubation, which means that calcium uptake took less time to reach plateau in this solution.

The rate of calcium influx in the PEG-1500 solution also showed an increase during the first 10–15 min of incubation. However, the rate of calcium uptake increased markedly (up to 0.952 $mM \times L\ cells^{-1} \times h^{-1}$) and reached a plateau only after 40 min of incubation.

A>2-fold decrease in turbidity (which indicates cell aggregation) was observed spectrophotometrically (at 650 nm band) in cells incubated in the PEG-1500 solution. No such aggregate formation was detected in the sucrose solution.

The data shown in Fig. 1 suggest that PEG's peroxides induced no LPO in the cells treated with the PEG solution. The slight increase (1.3 times as compared to the controls) in MDA concentration was observed only for the aged cells which were stored for more than 2 weeks in the buffer which contained glucose and anticoagulant (sodium citrate).

A decrease in the BTB binding parameter (n) was found for the cells suspended in PEG-1500 containing medium (n = 0.190 ± 0.022 for the controls and n = 0.098 ± 0.014 for the cells in the PEG-1500 medium). No difference in the values of n was observed when the cells pretreated with the PEG-1500 medium were washed twice with HIS medium to remove PEG-1500 (n = 0.190 ± 0.022 and n = 0.205 ± 0.025, correspondingly).

Since ^{1}H-NMR bands of carbohydrate groups of the polymer were not registered (Fig. 2) after the wash and destruction of the cells with 0.01% Triton X-100, we conclude that the effect of PEG-1500 on calcium influx was not due to the direct interaction of PEG with the cell membrane.

Discussion

Calcium influx experiments require Ca^{2+}-pump inhibition because of the low passive permeability of erythrocyte membranes and high functional activity of Ca^{2+}-ATPase. Calcium influx in 1 mM orthovanadate treated cells is linear over approximately 5 hours [25, 26]. Instead of orthovanadate, which directly and irreversibly inhibits the enzyme, metabolic inhibitors (e.g. inosine and iodoacetate) cause Ca^{2+}-ATP inhibition by decreasing intracellular ATP levels ($[ATP]_i$ decreases from 10^{-3} M to 10^{-6} M). Glucose presented in the solution and intracellular 2,3-DPG enables recovery of cellular ATP, thus the activity of the Ca^{2+}-ATPase may be restored during long incubation of the cells in glucose containing solutions.

The rates of Ca^{2+} influx ($\mu M \times L\ cells^{-1} \times h^{-1}$) in ATP-depleted human erythrocytes in isotonic physiological solution (HIS), sucrose (LIS) and polyethylene (glycol) 1500 (PEG-1500) solutions

Solution	Time of incubation, min	
	initial 10–15 min	next 30–40 min
physiological solution*	22.78 ± 7.61	19.31 ± 3.39
200 mM sucrose solution**	27.58 ± 8.96	9.13 ± 1.51
76 mM polyethylene (glycol) 1500 solution**	152.4 ± 50.57	952.3 ± 85.7

* The data of 4 independent experiments; ** The data of 3 independent experiments.

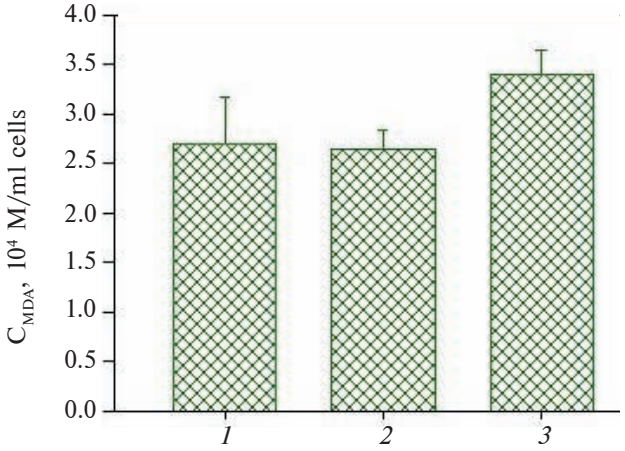

Fig. 1. Effect of PEG-1500 on LPO in human erythrocytes. X-axis: 1 – the control (erythrocytes in HIS); 2 – erythrocytes treated with 76 mM PEG-1500; 3 – "old" erythrocytes (stored more than 14 days at 4 °C in glucose and citrate containing medium) treated with 76 mM PEG-1500. Y-axis: MDA concentration (MDA), 10^{-4} M/ml cells.

The Ca^{2+} uptake in the ATP-depleted cells observed in the study may be characterised as 2 phase process, which includes: (I) rapid calcium accumulation in the cells (fully inhibited Ca^{2+}-pump); 2) slower accumulation with the plateau phase (Ca^{2+}-pump activity is partly recovered). Our data of initial rate of calcium uptake in the ATP-depleted cells incubated in the HIS (Table) are in a good agreement with published reports [27] for substrate-depleted cells loaded with another fluorescent calcium dye – quin-2 (16.2 ± 2.0 μM× L cells^{-1}×h^{-1}), but higher than that given in [28], which were obtained using ATP-depleted cells as well as the cells treated with vanadate (6.85 ± 0.38 μM× L cells^{-1}×h^{-1}). The effect observed may be due to the increase in Ca^{2+} buffering capacity of cells loaded with intracellular calcium chelators such as fura-2, quin-2, BAPTA etc.

Our results suggest that calcium uptake in ATP-depleted cells transferred to the isotonic LIS solution increases during the first minutes of incubation in LIS. We assume that the effect may be caused by a change in passive permeability of the membrane for calcium following membrane depolarisation in low ionic strength media [29]. The increase in negative surface potential on the outer membrane leaflet increases the cation concentration nearby the membrane facilitating calcium diffusion in the cells.

By contrast, during the second phase of Ca^{2+} entry, Ca^{2+} influx decreased in cells incubated in the LIS (Tabl.). A possible explanation is activation of Ca^{2+}-ATPase during incubation of the cells in the sucrose medium. The mechanism may involve increased interaction of Ca^{2+}-ATPase with calmodulin – Ca^{2+} complex when incomplete inhibition of the enzyme takes place. The rapid increase in calcium uptake during the first period, observed for the cells suspended in the LIS solution, elevates $[Ca^{2+}]_i$ and may accelerate calcium binding by calmodulin, the main intracellular calcium buffer [30].

The situation is more complicated in the case of cells treated with the PEG-1500. The data (Table) suggests that, despite the sucrose, PEG-1500 perturbs the membrane increasing membrane permeability for calcium and inhibits Ca^{2+}-ATPase.

Recently it was shown [10] that induction of LPO in sarcoplasmatic reticulum membranes led to abnormal Ca^{2+} transport. Two types of channels were induced by LPO: (i) the "passive" channels located in lipid bilayer of the membranes; (ii) the "active" ones which were found within lipoprotein complex of Ca^{2+}-pump.

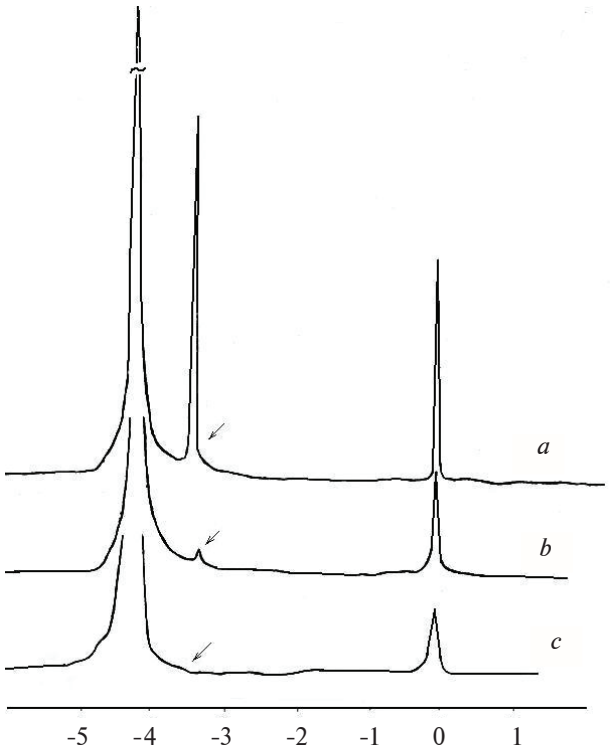

Fig. 2. ^{1}H-NMR spectra: a – 76 mM PEG-1500 solution; b – erythrocytes suspension (Ht=80%), treated with 76 mM PEG-1500 (1:1); c – the same cell suspension after the wash (3 times, 1200 g, 5 min, 22 °C) in HIS solution. The carbohydrate proton signal of PEG-1500 molecules is marked with the arrows. The signal from tetramethylsilan (TMS) was used as an internal standard.

Nevertheless there was found no induction of LPO by the peroxides of PEG-1500 (Fig. 1).

The experiments with the anion dye (BTB) demonstrated a significant fall in the dye sorption by the cells suspended in PEG-1500 containing medium as compared with the cells in physiological saline. Assuming that BTB binds to the cells proteins hydrophobically at physiological pH and its binding site involves a hydrophobic surface at the close distance to positively charged protein groups we concluded that PEG-1500 molecules might interact with the cells and/or screen some binding sites on the cell surface. Taking into account the existence of nonpolar groups of PEG-1500 molecule, intercalation of the molecules in the membrane bilayer was possibel. A subsequent local redistribution of the membrane proteins and lipids might alter the activity of Ca^{2+}-ATPase.

However the values of n for BTB binding to the cells pre-treated and then washed from PEG-1500 did not significantly differ from those in the physiological saline. Moreover we failed to detect membrane-bound PEG-1500. As shown in Fig. 2, there was no evidence for absorption of ^1H bands of carbohydrate groups of the polymer onto the cell surface following treatment with the PEG solution and lysis with Triton X-100. This result is in a good agreement with the published data [7], in which EPR spectra found no perturbation of tempamin-labelled sialic acid residues of the erythrocytes ghosts after polyethylene(glycol) removal. Thus an effect of PEG-1500 on calcium influx via direct interaction with the membrane was discounted. However, it remains possible that of the change physical and chemical properties of water environment by PEG might lead to aggregation and destabilisation of the membranes, which in their turn would change the membrane structure and osmotic pressure on the cells [6, 7, 18].

Since the osmolarities of the sucrose and PEG-1500 solutions used in the study were the same, an osmotic effect of PEG-1500 effect was also discounted. Moreover, it seems unlikely that the change in dielectric constant ($\varepsilon = 70.5$ for the PEG-1500 solution in comparison with $\varepsilon = 76.5$ for 150 mM NaCl saline [31] could play a significant role in destabilisation of the membrane structure and result in cation permeable pores. In this regard, it should be noted that the dielectric constant of the LIS solution was $\varepsilon = 74.8$, and no increase in $[Ca^{2+}]_i$ was observed in this medium.

Thus we assume that the effect of the PEG-1500 solution on Ca^{2+} influx is caused by the high dehydrative ability of the polymer. ^1H-NMR data [32] showed that there were 3 to 4 water molecules bound to each PEG unit. The amount of water was increased with the increase in PEG concentration (up to 16 water molecules per PEG monomer were structured in the 13% solution and nearly all water was bounded in the 38÷45% solution). It is well known that amphipathic molecules are able to regulate the activity of Ca^{2+}-ATPase by changing the access of the enzyme to water [1, 30]. Degradation of the hydrate environment of the enzyme leads to its inactivation. On the other hand, aggregation of the cells in the PEG-1500 solution and the change in hydrate surrounding of the protein-lipid complexes may vary membrane permeability for cations, thereby increasing $[Ca^{2+}]_i$.

Additionally, the involvement of Gardos channels in the increase of $[Ca^{2+}]_i$ after 20 min of incubation in the PEG-1500 cannot be completely discounted [9, 33, 34]. It has been shown that an increase in $[Ca^{2+}]_i$ from 1 to 100 μM (5 to 10 μM [33]) leads to significant membrane hyperpolarisation following increased K^+ permeability (Gardos channels activation) [34].

Thus the results of our study show that Ca^{2+} uptake by ATP-depleted human red blood cells depends on the composition of the incubation medium. In isotonic media where NaCl was completely substituted by impermeable for the cells sucrose or PEG-1500, a faster increase in $[Ca^{2+}]_i$ was observed during the first 10 min of incubation in the media, when compared with physiological saline. Long-term incubation (for 45 min) of the substrate-depleted cells in the low ionic strength solutions decreased the calcium uptake in the LIS solution and significantly increased it in the case of the PEG-1500 solution. Taking into account the data obtained here and those in the literature, the following mechanism of PEG-1500 effect on the cells is suggested: (i) membrane depolarisation and increase in C^{2+} influx; (ii) change in hydrate surrounding of Ca^{2+}-ATPase and its inactivation; (iii) aggregation of the cells with the pores permeable for cations formation; (iiii) dehydration of the cells due to the Gardos channels activation. Sucrose which has no ability to structure water molecules like that for PEG demonstrated no effect on the hydrate surrounding of Ca^{2+}-ATPase. The enzyme activation in the LIS solution may be due to incomplete reversible suppressing of the Ca^{2+}-pump activity in the substrate-depleted cells, which may be recovered and accelerated under the fast calcium uptake by the cells upon calmodulin binding with Ca^{2+}-ATPase.

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ДОСЛІДЖЕННЯ ПОТОКУ Ca^{2+} ДО ЕРИТРОЦИТІВ ЛЮДИНИ В ІЗОТОНІЧНИХ СЕРЕДОВИЩАХ ПОЛІЕТИЛЕНГЛІКОЛЮ (ПЕГ-1500) І САХАРОЗИ

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За допомогою флуоресцентного кальцієвого індикатору фура-2АМ досліджували вплив ізотонічних розчинів сахарози і ПЕГ-1500 на потоки кальцію до АТР-виснажених еритроцитів людини. Показано, що початкова швидкість потоку кальцію до еритроцитів, інкубованих у середовищах з низькою іонною силою, що вміщували 2 мМ $CaCl_2$, була вищою, ніж до клітин у фізіологічному середовищі. Після 20-хвилинної інкубації клітин у розчині ПЕГ-1500 спостерігалося 10-кратне посилення потоку кальцію до АТР-виснажених клітин, у той час як у сахарозному середовищі помічено зменшення швидкості потоку порівняно з клітинами у фізіологічному середовищі. Дані ^{1}H-ЯМР спектроскопії свідчать про відсутність прямої взаємодії молекул ПЕГ-1500 з мембраною еритроцита. Не виявлено також індукції пероксидного окислення ліпідів (ПОЛ) у мембранах еритроцитів під впливом ПЕГ-1500. Висловлено припущення, що зміна гідратного оточення Ca^{2+}-АТР-ази під впливом ПЕГ-1500 є первинною ланкою в ланцюзі порушень Ca^{2+}-транспортувальної функції клітин. Активація Ca^{2+}-АТР-ази в сахарозному середовищі, можливо, є наслідком неповного пригнічення активності Ca^{2+}-насоса в субстрат-виснажених клітинах і прискорення його роботи у разі зв'язування кальмодуліну з Ca^{2+}-АТР-азою в умовах швидкого накопичення кальцію клітинами.

Ключові слова: АТР-виснажені еритроцити, потік кальцію, сахароза, поліетиленгліколь 1500, фура-2.

ИССЛЕДОВАНИЕ ПОТОКА Ca^{2+} К ЭРИТРОЦИТАМ ЧЕЛОВЕКА В ИЗОТОНИЧЕСКИХ СРЕДАХ ПОЛИЭТИЛЕНГЛИКОЛЯ (ПЭГ-1500) И САХАРОЗЫ

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С помощью флуоресцентного кальциевого индикатора фура-2АМ исследовали влияние изотонических растворов сахарозы и ПЭГ-1500 на потоки кальция к АТР-истощенным эритроцитам человека. Показано, что начальная скорость потока кальция к эритроцитам, инкубированных в средах с низкой ионной силой, которые содержали 2 мМ $CaCl_2$, была выше, чем к клеткам в физиологической среде. После 20-минутной инкубации клеток в растворе ПЭГ-1500 наблюдалось 10-кратное усиление потока кальция к АТР-истощенным клеткам, в то время как в сахарозной среде отмечено уменьшение скорости потока по сравнению с клетками в физиологической среде. Данные ^{1}H-ЯМР-спектроскопии указывают на отсутствие прямого взаимодействия молекул ПЭГ-1500 с мембраной эритроцита. Не установлена также индукция пероксидного окисления липидов в мембранах эритроцитов при действии на них ПЭГ-1500. Высказано предположение, что изменение гидратного окружения Ca^{2+}-АТР-азы под влиянием ПЭГ-1500 является первичным звеном в цепи нарушений Ca^{2+}-транспортирующей функции клеток. Активация Ca^{2+}-АТР-азы в сахарозной среде, возможно, является следствием неполного угнетения активности Ca^{2+}-насоса в субстрат-истощенных клетках и ускорение его работы при связывании кальмодулина с Ca^{2+}-АТР-азой в условиях быстрого накопления клетками кальция.

Ключевые слова: АТР-истощенные эритроциты, поток кальция, сахароза, полиэтиленгликоль 1500, фура-2.

1. *Актуальные* проблемы криобиологии / Под ред. Н. С. Пушкаря, А. М. Белоуса. – Киев: Наукова думка, 1981. – 608 с.

2. *Терехов Н. Т., Петров М. М.* Эффективные трансфузионные эритроцитные среды. – Киев: Здоровья, 1990. – 165 с.
3. *Бабийчук Л. А., Землянских Н. Г.* // Проблемы криобиологии. – 1996. – № 4. – С. 32–38.
4. *MacDonald R. I.* // Biochemistry. – 1985. – **24**. – P. 4058–4066.
5. *Сенькович О. А., Розин В. В., Черницкий Е. А.* // Биол. мембраны. – 2001. – **18**, № 2. – С. 120–124.
6. *Boni L. T., Stewart T. P., Alderfer J. L., Hui S. W.* // J. Membrane Biology. – 1981. – **62**. – P. 65–70.
7. *Pratsch L., Herrmann A., Schwede I., Meyer H. W.* // Biochim. Biophys. Acta. – 1989. – **980**. – P. 146–154.
8. *Litwa M., Maggs A. M., Jin C. Z. et al.* // Mol. Membrane Biol. – 1997. – **14**. – P. 143–148.
9. *Руденко С. В., Пересецкая Н. М.* // Биохимия. – 1995. – **60**, № 7. – С. 1146–1154.
10. *Каган В. Е., Архипенко Ю. В., Ритов В. Б., Козлов Ю. П.* // Биохимия. – 1983. – **48**, № 2. – С. 320–330.
11. *Bookchin R. M., Lew V. L., Nagel R. L., Raventos C. J.* // J. Physiology. – 1980. – **312**. – P. 65.
12. *Bookchin R. M., Ortiz O. E., Shalev O. et al.* // Blood. – 1988. – **72**. – P. 1602–1607.
13. *Lew V. L., Ortiz-Carranza O. E., Bookchin R. M.* // J. Clinical Investigations. – 1997. – **99**. – P. 2727–2735.
14. *Romero P. J., Romero E. A.* // Cell Calcium. – 1999. – **26**. – P. 131–137.
15. *La Celle P. L., Kirpatrick F. H., Udkow M.* // Relation of altered deformability, ATP, DPG and Ca^{2+} concentration in senescent erythrocytes / Erythrocytes, thrombocytes, leucocytes. Stuttgart. – 1973. – P. 49–52.
16. *Дворянцев С. Н., Тимонин И. М., Петров В. В. и др.* // Биол. мембраны. – 1988. – **5**, № 10. – С. 1042–1050.
17. *Петруняка В. В., Северина Е. П.* // Там же. – 1992. – **9**, № 3. – С. 263–272.
18. *Esmann M., Hideg K., Marsh D.* // Biochemistry. – 1994. – **33**. – P. 3693–3697.
19. *Yingst D. R., Polasek P. M., Kilgore P.* // Biochim. Biophys. Acta. – 1985. – **813**. – P. 277–281.
20. *Ohno H., Shimidzu N., Tsuchida E. et al.* // Biochem. Biophys. Acta. – 1981. – **649**. – P. 221–228.
21. *Benaim G., de Meis L.* // FEBS Letters. – 1989. – **244**. – P. 484–486.
22. *Soldati L., Spaventa R., Vezzoli G. et al.* // Biochem. Biophys. Research Communications. 1997. – **236**. – P. 549–554.
23. Kucherenko Yu.V., Weiss E., Bernhardt I. // Bioelectrochemistry. 2004. 62. P. 127–133.
24. *Кучеренко Ю. В., Розанова Е. Д.* // Укр. біохім. журн. – 2001. – **73**, № 1. – С. 64–67.
25. *Lew V. L., Beauge L. A.* Transport across biological membranes / Eds. G. Giebisch, D. C. Toteson, H. H. Ussing. – Springer-Verlag. Berlin. – 1979. – **2**. – P.85–115.
26. *Hrabak H., Simonsen L. O.* // J. Physiol. – 1987. – **390**. – P. 95.
27. *McNamara M. K., Wiley J. S.* // Am. J. Physiol. – 1986. – **250**. – P. 26–31.
28. *Raess B. U., Keenen C. E.* // J. Membrane Biol. – 1996. – **151**. – P. 45–51.
29. *Bernhardt I., Hall A. C., Ellory J. C.* // J. Physiology. – 1991. – **434**. – P. 489–506.
30. *Орлов С. Н.* Кальмодулин. / Общие проблемы физико-химической биологии" (Итоги науки и техники ВИНИТИ АН СССР). Москва. – 1987. – **8**. – 212 с.
31. *Жилякова Т. А.* Температурозависимые изменения состояния воды в биологических мембранах по данным методов ЯМР и СВЧ-диэлектрометрии / Дисс. канд. биол. наук, Харьков. ИПКиК. – 1991. – 170 с.
32. *Баран А. А., Соломенцева И. М., Манк В. В., Куриленко О. Д.* // Доклады АН СССР. – 1972. – **207**. – С. 363–366.
33. *Орлов С. Н., Кравцов Г. М.* // Биохимия. – 1983. – **48**, № 9. – С. 1447–1455.
34. *Орлов С. Н., Покудин Н. И., Котелевцев Ю. В.* // Там же. – 1987. – **52**, № 8. – С. 1373–1386.

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