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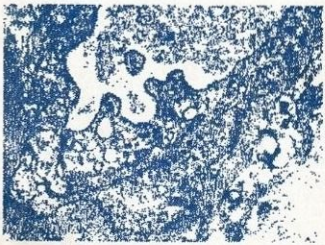
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PROBLEMS OF CRYOBIOLOGY



ON THE MECHANISM OF REGULATING BLOOD-BRAIN BARRIER PERMEABILITY OF THE COOLED BRAIN. REPORT 1 *G.A.Babiychuk, V.S.Marchenko, Yu.F.Pastukhov, A.V.Shilo, V.V.Lomako, I.I.Lomakin, L.N.Marchenko*

INVESTIGATION OF WATER-PROTEIN-LIPID INTERACTIONS IN RED BLOOD CELLS OF POIKILOTHERMIC ANIMALS *L.V.Tsymbal, S.V.Shovkoplyas, A.M.Belous*

STRUCTURE-FUNCTIONAL CHARACTER OF SOME INNER ORGANS OF ANIMALS FOLLOWING PROFOUND HYPOTHERMIA WITH PREMEDICATION BY METRANIDASOLE *V.F.Kozlova, T.N.Yurchenko*



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О.М.ДУНАЄВСЬКА, О.Р.ПАНТАЛЕР, Н.М.ШПАКОВА, В.А.БОНДАРЕНКО

ДЕЯКІ МОЖЛИВОСТІ ПІДВИЩЕННЯ СТІЙКОСТІ ЕРИТРОЦИТІВ ДО ХОЛОДОВИХ ТА ГІПЕРОСМОТИЧНИХ ВПЛИВІВ ПРИ ВИКОРИСТАННІ КАТІОННИХ АМФІПАТІВ

Інститут проблем кріобіології і кріомедицини НАН України, м. Харків

Представлені результати досліджень, які свідчать про те, що катіонні амфіпати: трифторперазин та хлорпромазин — захищають еритроцити при дії як гіперосмотичного, так і холодового шоку. Ступінь вияву отриманого ефекту залежить від температури та початкового рівня дегідратації клітин, так що при оптимальних умовах зазнають лізису тільки 10% еритроцитів. На основі аналізу фізико-хімічних властивостей вказаних сполук та існуючих у літературі даних про характер їх взаємодій з компонентами еритроцитарної мембрани обговорюються можливі механізми одержаного захисного ефекту.

О.Н.ДУНАЕВСКАЯ, Е.Р.ПАНТАЛЕР, Н.М.ШПАКОВА, В.А.БОНДАРЕНКО

НЕКОТОРЫЕ ВОЗМОЖНОСТИ ПОВЫШЕНИЯ УСТОЙЧИВОСТИ ЭРИТРОЦИТОВ К ХОЛОДОВЫМ И ОСМОТИЧЕСКИМ ВОЗДЕЙСТВИЯМ ПРИ ИСПОЛЬЗОВАНИИ КАТИОННЫХ АМФИПАТОВ

Институт проблем криобиологии и криомедицины НАН Украины, г.Харьков

Представлены результаты исследований, свидетельствующие о том, что катионные амфипаты: трифторперазин и хлорпромазин - способны оказывать протектирующее действие на эритроциты как при гиперосмотическом, так и при холодовом шоке. Степень выраженности полученного эффекта зависит от температуры и исходного уровня дегидратации клеток, так что при оптимальных условиях подвергаются лизису лишь 10% эритроцитов. На основе анализа физико-химических свойств указанных соединений и существующих в литературе данных о характере их взаимодействий с компонентами эритроцитарной мембраны обсуждаются возможные механизмы полученного защитного эффекта.

UDC 57.043:612.111:615.224

S.N.Fedosova, E.V.Kudokotseva

MODIFYING ACTION OF CURANTYL ON THE OSMOTIC SUSCEPTIBILITY OF RED BLOOD CELLS

Modifying action of curantyl on the susceptibility of red blood cells (RBC) of man and rat to the action of hyperosmotic stress was studied following pre-incubation of cells in NaCl solutions with varying tonicity at 0 °C and 37 °C. The action of curantyl increased the osmotic susceptibility of human RBC, while that of rats is reduced as compared with native cells independently of pre-incubation temperature. A supposition is due that a change in the RBC susceptibility to the action of hyperosmotic stress after a rise in the tonicity of the pre-incubation medium relates to the mechanism, modifying the interaction of a 4.1-band protein with capnophorine and other cytoskeletal and membrane proteins.

Currently solution of the urgent problems of transfusiology, in particular, maintenance of the structural, metabolic and functional integrity of RBC in the process of hypothermic storage, involves, mainly, modification of the composition of the rehabilitating and resuspending media.

Chemical compounds are proposed as one of the components of these media [3]. They are capable of inhibiting enzymes, which catalyse fission reactions of purine nucleotides, the latter being the only source of the ATP resynthesis in RBC [8]. The necessity for using these compounds arises from the fact that cooling and hypothermal storage result in the slowing down of the cell metabolism, in particular, of the main exchange path of RBC – glycolysis, which is due to high temperature factors of the catalytic reactions and a shortage of the required glycolytic substrates [10]. Slowing down of metabolism brings about depletion of the key products and intermediates, including the most important organic phos-

phates of the cell such as ATP. In turn, energetic depletion, while limiting numerous energy-dependent intracellular processes, causes impairments in the membrane structure-functional properties and, finally, results in the loss of the cell viability as a whole [8].

Some drugs are known to possess the ability for inhibiting the enzymes of purine degradation. In particular, one of them is coronarolytic curantyl, which, while inhibiting the activity of adenosine desaminase, prevents desamination of adenosine, required both for resynthesis of ATP and activation of adenylate cyclase [8, 10].

Until now systematic investigation of the actions of the substances of this class on the temperature and osmotic susceptibility of RBC has not been conducted.

Considering the above, the aim of the given work was to investigate the effect of curantyl on the osmotic susceptibility of man and rat RBC during cell dehydration in electrolyte solutions.

Red blood cells of Wistar rats, aged 12 months, and RBC of human donor blood of oup II, harvested at the Kharkov Regional Blood Transfusion Centre with a ZC iPK-76 preservative, were used as the object of investigation. Following removal of plasma the erythromass was thrice washed by physiological saline, containing 150 mM NaCl, 10 mM Na-phosphate buffer, pH 7.4. All solutions were prepared with Na-phosphate buffer, pH 7.4. RBC were treated with curantyl during cell incubation in NaCl physiological solution, containing 0.05 mM of this preparation, for 10 min at 37 °C.

For studying the effect of the pre-incubation medium tonicity on the RBC susceptibility to the action of hyperosmotic stress the cells were incubated in the NaCl solutions with different tonicity (0.15-1.05 M) for 10 min at 0, 37 °C. After that the aliquotes of 0.1 ml of each sample were transferred into 0.9 ml of 4.0 M NaCl for 10 min at 0, 37 °C. Stability of the RBC cytoskeleton to mechanical action was assessed using a method described elsewhere [9], in which the RBC suspension following pre-incubation in the NaCl solutions at 0, 37 °C was pumped 6 times through a medical needle with the diameter of 0.3 mm. After completion of these procedures the samples were centrifuged with an OPN centrifuge for 3 min at 3,000 rpm, and the supernatant absorption was determined spectrophotometrically with -543 nm. The yield of hemoglobin was calculated in proportion to a 100%-hemolysis.

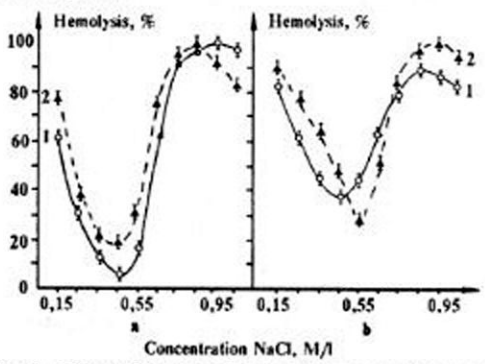


Fig. 1. Effect of curantyl and original osmotic conditions of human RBC incubation in solutions of NaCl (0.15-1.05 mol/l) at 0 °C (*a*) and 37 °C (*b*) on the level of cell hemolysis after transfer into 4.0 mol NaCl: *1* – native human RBC, *2* – curantyl-treated human RBC.

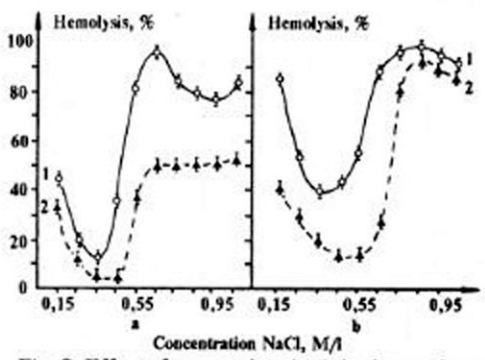


Fig. 2. Effect of curantyl and original osmotic conditions of rat RBC incubation in solutions of NaCl (0.15-1.05 mol/l) at 0 °C (*a*) and 37 °C (*b*) on the level of hemolysis after transfer into 4.0 mol NaCl: *1* – native rat RBC, *2* – curantyl-treated rat RBC.

The authors have found that when increasing tonicity of the pre-incubation medium up to 0.45 M NaCl the stability of human and rat RBC to subsequent transfer of cells into 4.0 M NaCl increases (Figs. 1 and 2), and the minimum lysis of human RBC is observed after pre-incubation in 0.35-0.55 M NaCl, and in rat RBC – in 0.25-0.45 M NaCl. The temperature of the pre-incubation medium significant-

ly affects cell susceptibility to subsequent alteration of the medium osmolarity: RBC pre-incubation at 0 °C reduces the level of cell injury following subsequent action of hyperosmotic stress. Despite the absence of significant differences in the susceptibility of the human and rat RBC to hyperosmotic stress, treatment of cells with curantyl imposes species-dependent specificity on the response of cells to a change in the medium osmolarity. The osmotic susceptibility of the rat RBC is reduced (Fig. 2), while the susceptibility of human RBC to hyperosmotic stress increases as compared with the osmotic susceptibility of the native RBC (Fig. 1).

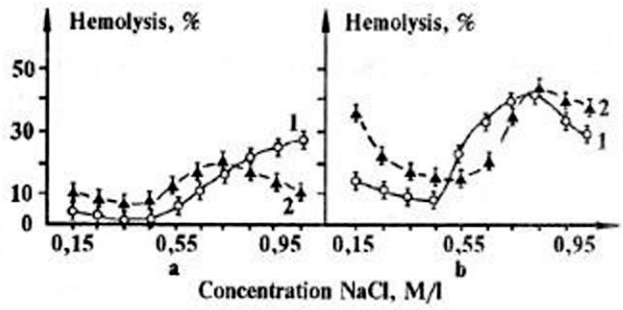


Fig. 3. Effect of curantyl on the level of human RBC hemolysis during mechanical treatment following cell pre-incubation in solutions of NaCl with varying tonicity at 0 °C (*a*) and 37 °C (*b*): *1* – native human RBC, *2* – curantyl-treated human RBC.

It follows from the data submitted that the susceptibility of RBC to hyperosmotic stress is determined by the original incubation conditions, which provide for partial cell dehydration. It may be supposed that the major part in the alteration of the RBC susceptibility to a change in the medium osmolarity is played by the altered structural state of a «cytoskeleton-membrane» complex, since partial dehydration of RBC results in the concentration of the cytoskeletal structural elements in the membrane-adjacent layer, which, in turn, may affect the nature of their interaction with the membrane [2].

With the aim of checking this assumption the authors have studied mechanical susceptibility of RBC during their dehydration in the NaCl solutions with various tonicity at 0.37 °C.

A local minimum of human RBC lysis following mechanical treatment in the presence of 0.35-0.45 M NaCl, apparently, reflects the cytoskeleton structural organization and a change in the nature of its interaction with membrane, which coincides with the range of the minimum susceptibility of human RBC to hyperosmotic stress (Fig. 3, *1*). The susceptibility of the curantyl-treated human RBC to mechanical treatment at 37 °C increases after pre-incubation of cells in 0.15–0.45 M and 0.85-1.05 M NaCl, while pre-incubation in 0.55-0.75 M NaCl reduces it as compared with the mechanical susceptiblity of the native RBC, which nicely correlates with a change in the susceptibility of human RBC to hyperosmotic stress under the action of curantyl. The nature of changes in the mechanical susceptibility of human RBC following curantyl treatment correlates with the alteration in the osmotic susceptibility of these cells after pre-incubation in the solutions of NaCl at 0 °C.

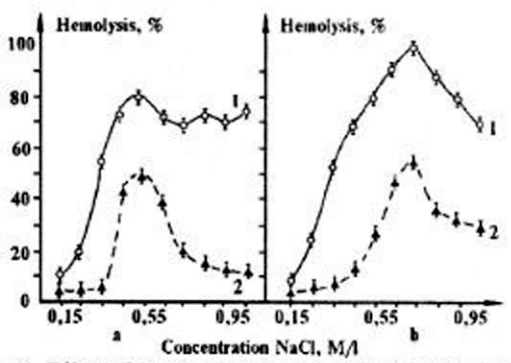


Fig. 4. Effect of curantyl on the level of rat RBC hemolysis during mechanical treatment following cell pre-incubation in solutions with varying tonicity at 0 °C (*a*) and 37 °C (*b*): *1* – native rat RBC, *2* – curantyl-treated rat RBC.

The susceptibility of rat RBC to mechanical treatment is much higher as compared with that of human RBC (Fig. 3, *4*).

Rat RBC, treated with curantyl, become more resistant to mechanical treatment as compared with the native cells, which correlates with a reduction in the

sensitivity of rat RBC to hyperosmotic stress under the action of curantyl (Fig. 2, *4*).

The results obtained support the assumptions [2] that the structural basis of the mechanism, which controls osmotic susceptibility of RBC during dehydration in the electrolyte solutions, is the cytoskeleton.

A working hypothesis, which explains a varying modifying action of curantyl on the osmotic and mechanical susceptibility of RBC of man and rat, may be nicely illustrated on the basis of the data [7] on the susceptibility of RBC to mechanical treatment to be due to the interaction of the band 4.1 protein with glycophorine C. Judging from the knowledge [6] on the absence of glycophorins in rat RBC, high sensitivity of rat RBC to mechanical treatment may be easily explained from this point of view.

It is common knowledge that osmotic shrinking of RBC of the rat, but not of the man, results in the activation of the polyphosphoinositide cycle [5], which brings about formation of inositol triphosphate. The latter, when entering the cell cytoplas. causes a release of the Ca^{2+} ions from the intracellular protein stores, thus inactiva g adenylate cyclase, which relates in the switching-off of all the cAMP-dependent processes in the cell. Since curantyl, while inhibiting adenosine desaminase, increases the content of adenosine in the cell, then after treating rat RBC with this preparation, apparently, adenylate cyclase is not inhibited in the process of hyperosmotic shrinking, and the cAMP-dependent processes of the cytoskeletal protein phosphorylation, in particular, of proteins of the bands 4.1, 4.5, 2.1, are activated, which is manifested by a rise in the stability of rat RBC.

Treatment of human RBC with curantyl increases the pool of ATP in the cell, which is accompanied by inhibition of the main phosphoglycerate branch of glycolysis, thus faclitating a rise in the level of 2.3-DPG [8]. An increase in the concentration of 2.3-DPG brings about the impairments in the link of the band 4.1 protein with glycophorine C due to a predominant binding of the band 4.1 protein with capnophorine [7], which, in turn, results in the dissociation of the band 4.1 protein-spectrin-actin complex [7], which is manifested as a reduction in the mechanical stability of human RBC under the effect of curantyl.

Thus, it may be supposed that a change in the RBC susceptibility to hyperosmotic stress after a rise in the tonicity of the pre-incubation medium relates to the mechanism, which is based on the modification of the interaction of the band 4.1 protein with capnophorine and other proteins of the cytoskeletal membrane.

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С.М.ФЕДОСОВА, О.В.КУДОКОЦЕВА

МОДИФІКУЮЧИЙ ВПЛИВ КУРАНТИЛА НА ОСМОТИЧНУ ЧУТЛИВІСТЬ ЕРИТРОЦИТІВ

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Вівчався модифікуючий вплив курантила на чутливість еритроцитів людини та щура до дії гіперосмотичного шоку після прединкубації клітин в розчинах NaCl з різною тонічністю при 0 та 37 °С. Виявлено, що під впливом курантила осмотична чутливість еритроцитів людини збільшується, а щура зменшується у порівнянні з інтактними клітинами, незалежно від температури прединкубації. Припускається, що зміна чутливості еритроцитів до дії гіперосмотичного шоку при збільшенні тонічності середовища прединкубації пов'язана з механізмом модифікації взаємодії білка смуги 4.1 з капнофорином та другими білками мембран і цитоскелета.

С.Н.ФЕДОСОВА, Е.В.КУДОКОЦЕВА

МОДИФИЦИРУЮЩЕЕ ВЛИЯНИЕ КУРАНТИЛА НА ОСМОТИЧЕСКУЮ ЧУВСТВИТЕЛЬНОСТЬ ЭРИТРОЦИТОВ

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Изучалось модифицирующее влияние курантила на чувствительность человека и крысы к действию гиперосмотического шока после прединкубации клеток в растворах NaCl с различной тоничностью при 0, 37 °С. Показано, что под влиянием курантила осмотическая чувствительность эритроцитов крысы уменьшается, а человека – увеличивается, по сравнению с нативными клетками, независимо от температуры прединкубации. Полученные результаты обсуждаются с позиции влияния курантила на внутриклеточное содержание АТФ и цАМФ, участвующих в регуляции физико-химического состояния белков цитоскелета в клетке при действии осмотически активных соединений. Предполагается, что изменение чувствительности эритроцитов к действию гиперосмотического шока при увеличении тоничности среды прединкубации связано с механизмом модификации взаимодействий белка полосы 4.1 с капнофорином и другими белками мембраны и цитоскелета.

CRYOPRESERVATION OF BIOYSTEMS

UDC 57.043

T.F.Stribul, S.M.Tyumchuk, N.N.Milshina

STIMULATING ACTION OF LOW TEMPERATURE FREEZING OF MAIZE SEEDS ON THEIR GERMINATION

The authors have studied the effect of the conditions of freeze-thawing of maize seeds, possessing different levels of humidity, on their survival rate after cryoexposure. The protocols of cryopreservation and values of the optimal humidity of seeds have been elaborated, the application of which provides for stimulation of intensity of growth of shoots, germinated from cryopreserved seeds. A mehanism of stimulating action of low temperature pre-treatment of seeds on the process of their subsequent germination has been proposed.

A predominant factor in the process of plant seed cryopreservation is their humidity. The seeds of most agricultural cultures with the original humidity of 8–20% demonstrate certain stability to the action of low temperatures, still maintain-