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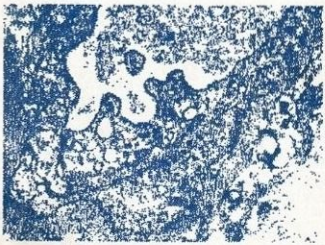
ISSN 0233–7673

NATIONAL ACADEMY OF SCIENCES OF THE UKRAINE
DEPARTMENT OF BIOCHEMISTRY, PHYSIOLOGY AND MOLECULAR BIOLOGY

INSTITUTE FOR PROBLEMS OF CRYOBIOLOGY AND CRYOMEDICINE

1995

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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SCIENTIFIC AND THEORETICAL JOURNAL

FOUNDED IN 1985
QUARTERLY PUBLISHED

1995

PROBLEMS OF CRYOBIOLOGY

(PROBLEMY KRIOBIOLOGII)

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National Academy of Sciences of the Ukraine, "Problems of Cryobiology", 1995

Е.З.ЕМІРБЕКОВ, С.П.ЛЬВОВА, М.К.КЛІЧХАНОВ

БІОХІМІЧНІ ЗМІНИ У КРОВІ ПРИ ШТУЧНІЙ І ПРИРОДНІЙ ГІПОТЕРМІЇ І ЗИМОВІЙ СПЛЯЧЦІ ССАВЦІВ

НДІ біології Дагестанського університету, м. Махачкала

Зроблено огляд даних літератури і власних результатів по біохімії крові у динаміці гіпотермії і зимовій сплячці ссавців. Автори приходять до висновку про помітні відмінності в біохімічних показниках крові і фізико-хімічних характеристиках ерітроцитів при штучному і фізіологичному зниженні температури тіла.

Э.З.ЕМИРБЕКОВ, С.П.ЛЬВОВА, М.К.КЛИХАНОВ

БИОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ В КРОВИ ПРИ ИСКУССТВЕННОЙ И ЕСТЕСТВЕННОЙ ГИПОТЕРМИИ

НИИ биологии Дагестанского университета, г.Махачкала

Представлен обзор данных литературы и собственных результатов по биохимии крови в динамике гипотермии и зимней спячки млекопитающих. Авторы приходят к заключению о наличии заметного различия в биохимических показателях крови и физико-химических характеристиках эритроцитов при искусственном и физиологическом снижении температуры тела.

UDC 57.043.612.111

O.N.Dunayevskaya, E.R.Pantaler, N.M.Shpakova, V.A.Bondarenko

SOME POSSIBLE METHODS OF INCREASING RED BLOOD CELL STABILITY TO THE ACTION OF COLD AND OSMOTIC EFFECTS AFTER APPLICATION OF CATION AMPHIPATES

There are submitted the results of investigations, testifying to the fact that cation amphipates, namely: trifluoroperazine and chlorpromazine – are capable of exercising a protective action on red blood cells (RBC) during both hyperosmotic and cold shock. The extent of manifestation of the observed effect depends on temperature and the value of the original level of cell dehydration, and only 10% of RBC are affected by lysis under optimal conditions. On the basis of the analysis of the physico-chemical properties of the above compounds and review of the data currently available on the nature of their interaction with the components of RBC membranes the authors speculate on the possible mechanisms of the protective effect observed.

A complicated set of unfavorable factors affects biological systems following a change in their environmental temperature and osmotic conditions [1]. The basic factors of low-temperature preservation, capable of inflicting impairments in the structure and function of biological systems, are high salt concentrations, which are developed during freezing-out of frozen water, and temperature shift.

To evaluate the contribution of the alterations in the osmotic and temperature environmental conditions into the resulting damaging effect during freezing use may be made of the experiments, modelling the action of osmolarity and temperature shift at positive temperatures. The action of hyperosmotic stress after transfer of cells into highly concentrated solutions of the electrolyte (4.0 M NaCl) simulates hyperosmotic stress, and the effects of hyperosmotic stress correlate with freezing effects [3]. Cold shock is observed after cooling from 37 °C down to 0 °C following pre-exposure of cells in hypertonic solutions of salts or non-electrolytes [10], and the original range of tonicity, within which the cells are sensibilized to cooling, coincides with the one during hyperosmotic stress. Thus, original osmotic conditions, under which the cells reside, are the factor, which determines the extent of their sensitivity to cold and osmotic stress.

Since the main target of the damaging action of hyperosmolarity is the plasma membrane [1, 9], one may suppose that temperature and osmotic sensitivities of

the cells will be altered during modification of the membrane physico-chemical properties. A significant modifying action on the components of the biological membrane [18] and, hence, on the cold and osmotic sensitivities of the cells are exercised by aliphatic compounds [5, 6].

The aim of the given work was to study a modifying action of cation amphipates (trifluoroperazine, chlorpromazine), which are capable of distribution into the inner membrane monolayer, on the susceptibility of red blood cells (RBC) to cold shock and hyperosmotic stress.

Red blood cells were isolated from donor blood of the A group. Following removal of plasma RBC were twice washed by physiological saline (0.15 M NaCl, 5 mM phosphate buffer, pH 7.4). The suspension obtained (hematocrit was 50%) was stored for not more than 2 hours at 0 °C.

With the aim of sudying the influence of hyperosmotic stress RBC were pre-incubated in media with varying content of sodium chloride (from 0.15 to 1.0 M) at definite temperatures for 2 min with subsequent isothermic transfer into a hypertonic NaCl solution (4.0 M) for 5 min (finite hematocrit was 0.4%).

For development of cold shock the samples, pre-incubated at 37 °C for 10 min, were cooled down to 0 °C (time of exposure was the same). The temperature was controlled by a copper-drop thermoelectric transformer.

Cell modification invoved application of trifluoroperazine in a 3×10^{-5} M concentration and chlorpromazine in a $7 \cdot 10^{-5}$ concentration.

Following completion of the incubation period the samples were centrifuged, and supernatant absorption was determined spectrophotometrically at a wavelength of 543 nm. Absorption of the sample, to which the detergent X-100 was added (0.1%), was considered equal to 100%-absorption.

Hypertonic stress, which is developed following transfer of RBC in the media with high salt concentrations, exercises a damaging action on cells depending on the osmotic and temperature conditions prior to and after transfer. It follows from the data in Fig.1 that the extent of injury of RBC, pre-exposed in the physiological (according to its tonicity) saline (0.15 M NaCl), achieves 70-90% under hyperosmotic stress in the media, which contain 4.0 M NaCl, and at 0 °C the cells experience lysis to a lesser extent as compared with 22 and 37 °C.

Proceeding from the fact that chemical compounds from the group of phenothiazines, to which trifluoroperazine belongs, are calmodulin inhibitors, protecting RBC during hypertonic stress [6] and significantly increasing their stability to the action of cold shock, which is developed after cooling of cells from 37 °C to 0 °C [5], it seems expedient to investigate the effect of trifluoroperazine on the RBC susceptibility to a transfer into highly concentrated solutions of the electrolyte (4.0 M NaCl), i.e. on the resistance of cells to hyperosmotic stress.

Trifluoroperazine is a positively charged derivative of phenothiazines, changing the shape of RBC along a "discocyte-stomatocyte" pathway. The said aliphatic compound is capable of crossing the plasma membrane and getting distributed at the interface of the cytoplasmic surface of bilayer and cytoskeletal proteins. As proposed by a bilayer couple hypothesis [7], insertion of the amphipate increases the area of the inner monolayer with respect to the outer one, which, in the long run, underlies formation of stomacytogenic forms of RBC. Recently the data have become available, which testify to the fact that these amphipatic compounds are mainly distributed in the cytoplasm [7], thus affecting the state of the cytosolic components; however, subsequent studies make these results doubtful [14, 15].

Application of trifluoroperazine permits to modify the sensitivity of RBC to hyperosmotic stress (Fig. 1). It follows from Fig. 1 that trifluoroperazine, after achieving a concentration, which is particular for every studied temperature modes, protects cells agains injury following the action of high salt concentrations, and a further rise in the amphipate concentration is accompanied by this effect becoming the opposite and an increase in cell lysis. The temperature strongly

determines the nature of RBC protection in the presence of trifluoroperazine and a corresponding range of the amphipate concentrations, within which cell resistance to the action of unfavorable factors increases. A rise in the temperature from 0 to 37 °C in the optimum concentration range shifts from $8 \cdot 10^{-6}$ $2 \cdot 10^{-5}$ M at 0 °C to $2 \cdot 10^{-5}$ $4 \cdot 10^{-5}$ M and $3 \cdot 10^{-5}$ $5 \cdot 10^{-5}$ M at 22 and 37 °C, respectively. It should be noted that under optimum concentration of trifluoroperazine and physiological temperature the minimum level of cells sensitivity achieves 10%, while at 0 °C – 35-40%. Thus, if the extent of protection in the presence of trifluoroperazine at 0 °C reaches 20-25%, then at higher temperatures it achieves the level of 70-80%.

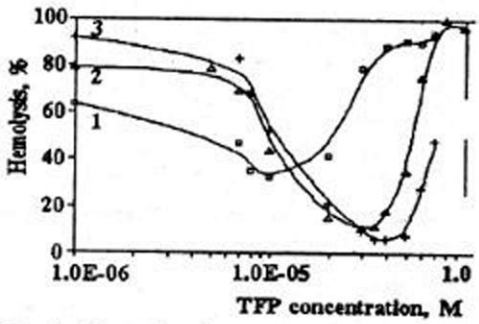


Fig. 1. Efect of various concentrations of trifluoroperazine (TFP) and temperature on the level of RBC hemolysis upon transfer into 4 M NaCl: *1* – 0 °C, *2* – 22 °C, *3* – 37 °C.

The data obtained testify to the fact that under different temperature modes there are developed different states of the cells, varying in their ability to counteract a damaging action of the stress factor, on one hand, and to a modifying action of trifluoroperazine, on the other. At physiological temperature a protective action of trifluoroperazine is pronounced much higher.

Apparently, RBC modification in the presence of trifluoroperazine initiates a number of changes in them, which underlies a rapid rise in the cell resistance to the damaging environmental factors, in particular, to hyperosmotic stress, which is developed in 4.0 M NaCl.

Preliminary partial dehydration of cells is also accompanied by the alterations in the structures of the membrane and cytoskeleton, facilitating formation of such cells states, which are characterized by an increased resistance to stress factors. It is common knowledge, in particular, that pre-dehydration of RBC significantly alters cell sensitivity to both mechanical and hypertonic stresses in 4.0 M NaCl [2].

It follows from the data, submitted in curve *1* in Fig. 2, that the range of the concentrations of sodium chloride, the incubation in which imposes resistance to the action of high electrolyte concentrations, is relatively narrow. It is thought that resistance of RBC, pre-treated in the media, containing 0.4-0.5 M NaCl, to the action of hyperosmotic stress after transfer of cells into 4.0 M NaCl, is due to the changes in the volumes of the cells, which are responsible for the formation of the stable state [2, 3]. Elsewhere [4] it has been reported that cell resistance to transfer into the media with a high content of electrolyte is strongly determined by the nature of the association-dissociation of a band 3 protein with the cytoskeleton.

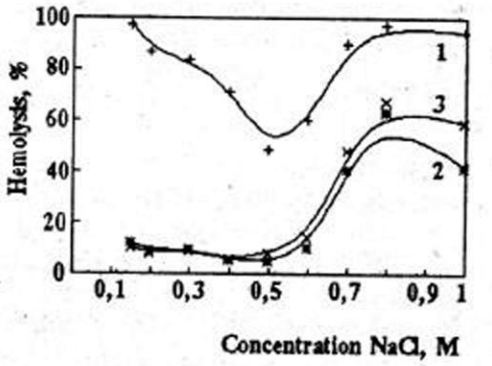


Fig. 2. Effect of cation amphipates and predehydration on the level of RBC hemolysis upon transition into 4 M NaCl: *1* – control; *2* – trifluoroperazine (TFP) (3×10^{-5} M); *3* – chlorpromazine (CPR) (7×10^{-5} M).

For correction of the RBC states at the stages, preceding cell transfer into 4.0 M NaCl, and taking into account the data in Fig. 1, the authors used trifluoroperazine in a $3 \cdot 10^{-5}$ M concentration and partial dehydration of cells. The results obtained, which reflect a combined effect of partial dehydration and trifluoroperazine on cell resistance to transfer into 4.0 M NaCl, are submitted in Fig. 2 (curve *2*). It is clear that application of the amphipate against partial cell dehydra-

tion in the electrolyte media permits to increase RBC stability to the action of stress factors within the studied range of sodium chloride concentrations.

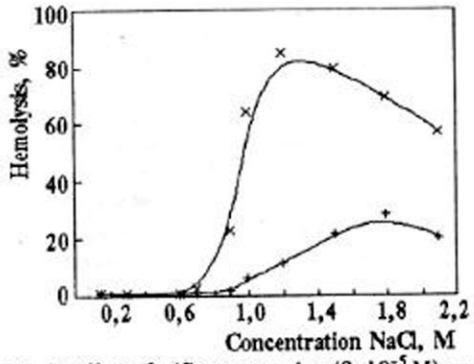


Fig. 3. Effect of trifluoroperazine (3×10^{-5} M) and pre-dehydration on the level of RBC hemolysis during cold shock: *1* – control; *2* – trifluoroperazine.

Human RBC are known to acquire susceptibility to cooling in the temperature range of 37–0 °C following pre-exposure in hypertonic solutions of salts and non-electrolytes [10]. It follows from the data, submitted in Fig. 3, that the development of cell injury during cooling from 37 °C to 0 °C is identified in the media with the NaCl content above 0.75 M, reaching maximum values in the presence of 1.2 M sodium chloride. This coincides with the osmolarity range, within which the membrane barrier function is disturbed and channels for leakage of K^+ ions are formed [5]. In the case of the cells, modified by trifluoroperazine in a $3 \cdot 10^{-5}$ M concentration, there is observed a considerable rise in resistance to the action of cold shock as compared with native RBC (Fig. 3).

It should be noted that another representative of the group of phenothiazines – chlorpromazine – also protects cells both from shock [5] and hyperosmotic stress (Fig. 2, curve *3*). A protective action of the said amphipate is realized when applied in the concentration of $7 \cdot 10^{-5}$, i.e. to obtain a maximum protective effect against the action of the damaging factors one needs a concentration of chlorpromazin to be by 2-fold higher as compared with trifluoroperazine. The given amphipatic compounds differ in some physical-chemical characteristics. For instance, the distribution factor in a water-octanol system is higher for trifluoroperazine as compared with chlorpromazine, which is indicative of a higher manifested hydrophobicity of the former compound [19]. That is why trifluoroperazine is easier distributed in the membrane, and identification of a similar membrane event requires it in a lower amount [19]. Being the inhibitor of calmodulin and capformer, trifluoroperazine possesses stronger action as compared with chlorpromazine [6, 11]. Inhibition of Ca-ATPase involves a concentration of trifluoroperazine, which is by 2.4-fold lower as compared with chlorpromazine [8]. At the same time, the concentrations of the said amphipatic compounds, which are required for cell protection against the action of hypotonic stress, differ by 8-fold [16].

Thus, trifluoroperazin as well as chlorpromazine, exercises protective action on RBC both against hyperosmotic stress and cold shock. In other words, the given amphipates facilitate triggering of the reserve abilities of the cell and formation of its stable state, permitting to tolerate transition into unfavorable environmental conditions. Since the said substances perform multiple action on the cells, it is difficult to separate a single "node" or structure, providing for the RBC resistance to a stress. However, judging from the physico-chemical properties of amphipatic compounds, their ability to affect the process of closing of RBC ghosts [12, 13], as well as inability to alter the shapes of RBC, in which the cytoskeleton is dissociated from the membrane bilayer due to selective fusion of ankyrine [9], two possible mechanisms may be identified, which provide for protective action of trifluoroperazine and chlorpromazine. One of the mechanisms, apparently, relates to influence of the above substances on the nature of association of protein cytoskeleton with the plasma membrane. A predominant localization of cation amphipates in the inner membrane monolayer may be a pre-requisite to weaking of interactions at the interface, separating the membrane plasma surface from the peripheral protein network. This weakens the action of factors, providing for

stabilization of defects, which are developed both after temperature shift under cold shock conditions and the action of hyperosmotic stress in 4.0 M NaCl.

Another mechanism of protective action of trifluoroperazine and chlorpromazine may consist in the direct influence of the above substances on the processes of growth and stabilization of bilayer defects, which are developed under the action of the unfavorable environmental conditions. Since negative charges on the pore walls may considerably contribute to the control over its dimensions and life period, it seems probable that positively charged amphipatic compounds will facilitate "healing" of defects, while neutralizing the charges on their boundaries.

The authors plan to conduct further studies with the aim of getting profound knowledge on the structural grounds of the observed protective effect of phenothiazines.

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Institute for Prblems of Cryobiology and Cryomedicine of the National Academy of Sciences of the Ukraine, Kharkov

Accepted on July 9, 1994

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-