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Use of Plasmalemma Reserve by Lymphocytes for Osmotic Stress Tests *In – vitro.*

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ABSTRACT

Atomic force spectroscopy method revealed the relationship between the elastic properties of membranes and the use intensity of additional membrane structures in the process of lymphocytes volume regulation from healthy donors with the osmolarity medium decrease. The increase of lymphocytes rigidity by 1.5 times under the adrenaline load resulted in the cell use intensity decrease by almost 2 times in relation to the relative membrane reserve. However, the reduction in stiffness under the calcium load influence by 1.5 times creates the conditions for intensive use of the cell membrane reserve at the regulation of volume under hypo-osmotic stress conditions.

Keywords: membrane reserve, lymphocytes, erythrocytes, atomic force spectroscopy, osmotic load.

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INTRODUCTION

The membrane reserve serves as morphological basis for the volume homeostasis maintenance of animal cells [1] presented by plasmalemma formations (folds, filopodium, lamelopodium etc.). The use of additional membrane structures allows to make rapid cell fluctuations of volume during the implementation of physiological functions. In particular, the membrane reserve is used by lymphocytes during the pseudopodia formation during migration [2], phagosome formation [3] during the processes of deformation during the blood cells passage through the capillaries [4]. Purpose of article: to establish the relationship between the elastic properties of cells and the intensity of the membrane reserve by these cells at osmotic load tests in vitro.

METHODS

The lymphocytes of 100 healthy donors were chosen as the object of study. The human blood was obtained by venipuncture. The blood was collected into vacuum tubes Vacuette K3E. The relative membrane reserve of cells was evaluated in tests with hypo-osmotic stress in vitro, according to the procedure published in earlier studies [5]. In order to identify the relationship between elastic properties and the usage intensity of standby pools osmotic by the cells of osmotic tests in vitro, the performed study utilized the functional load method comprising the cell suspension incubation with adrenaline, obzidan, calcium chloride and verapamil. The adrenalin load was performed by the incubation of 30 mcl of cell suspension in 150 mcl of Hank's medium containing 10^{-9} mmol/l of adrenaline for 15 min. The sample with obzidan was performed by the incubation of 30 mcl of suspension in 150 mcl of Hank's medium containing 10^{-9} mmol/l of obzidan for 15 min. The calcium load was performed by the incubation of 30 mcl of cell suspension in 150 mcl of Hank's medium containing 10^{-6} mmol/l of Ca^{2+} for 15 min. The sample with verapamil was conducted by the incubation of 30 mcl of cell suspension in 150 mcl of Hank's medium containing 10^{-6} mmol/l of verapamil for 15 min. Human lymphocytes were incubated in an incubator at the temperature of 37 C°. Upon the completion of the functional load time, the samples were centrifuged for 5 min at 1500 rev/min, and the supernatant liquid was removed and the osmotic tests were performed *in vitro*. Given that cytoskeleton elements determine the elastic properties of the cell surface the Young's modulus was measured before and after the load function for the performed study. The Young's modulus of the blood cells, quantitatively characterizing the membrane rigidity was measured by force spectroscopy using an atomic force microscope of Integra Vita. The preparation of samples and the measurement procedures for conducting force spectroscopy were performed as described in the article [6]. The obtained experimental data were statistically processed. The significance of differences was determined by using Student's t criterion.

MAIN PART

In terms of adrenalin load the Young's modulus of the blood cells was significantly increased by 57% ($p<0.05$), while under the influence of the non-selective β-adrenoceptor, obzidan blockers the stiffness of lymphocytes was decreased by 20% ($p<0.05$) compared to control (Table).

Table

Young's modulus and relative lymphocyte membrane reserve

Samples	Young's modulus, μPa	Relative membrane reserve, mcm^{-1}	
		cell	nucleus
Plasma (control)	3,50±0,20	0,17±0,001	0,09±0,001
Adrenalin	5,49±0,37*	0,07±0,001*	0,09±0,002
Obzidan	2,79±0,29*	0,03±0,001*	–
Alcium load	2,31±0,26*	0,11±0,002*	0,16±0,002*
Verapamil	2,55±0,41*	0,01±0,001*	–

** - Statistically significant differences between the values in load samples compared with the control by the Student's criterion at p <0.05.*

The use of the relative membrane reserve, by the lymphocytes in samples incubated with adrenaline, decreased by 59% ($p<0.05$) compared with the control, while obzidan samples demonstrated 82% ($p <0.05$) of decrease. On the level of nucleus reaction the divergent reactions were revealed using membrane reserve. So under β-adrenoceptor activation with adrenaline the relative membrane reserve activated by nucleus was the same compared to the control, and the nucleus was not involved in osmoregulatory reactions within β-adrenoceptor blockade conditions. Under the influence of the calcium load, as well as in the samples with

verapamil the Young's modulus of lymphocytes decreased by 34% and 27% ($p < 0.05$) compared to the controls (see the table). In the samples incubated with calcium chloride, the use of the relative membrane reserve in the cell hypotonic medium has decreased by 35% ($p<0.05$), and with the nucleus the use was increased by 77% ($p < 0.05$) compared with control. The samples incubated with verapamil, the intensity of plasmalemma surface stocks use by cell decreased by 94% ($p<0.05$) compared with control, while the nucleus was not involved in osmoregulatory responses.

CONCLUSION

Under the influence of adrenaline load, the stiffness of lymphocytes from healthy donors increased, which has limited their use of a large part of the surface membrane reserve in hypotonic medium as compared with the calcium load. The literature has the data on the cytoskeleton stiffness effect on the membrane tank size. It has been shown that the microtubule density increase improves microtubule cytoskeleton rigidity and the amount of membrane reserve available for use is reduced [7]. However, the elastic properties of cells are not the only limiting factor influencing the use of membrane reserves in physiological reactions. This conclusion is made on the basis of the data concerning the use of membrane reserve by the cells under the elements blockade conditions of the calcium and adenilate cyclase signal ducts. Thus, despite the reduction of cell stiffness under the influence of obzidan and verapamil the intensity of membrane reserve use by cells is also reduced. This fact is probably related to the concentration of intracellular signaling molecules. According to the literature, obzidan and verapamil are able to reduce the intracellular Ca^{2+} ions concentration [8, 9], the content of which determines the depolymerization of actin filaments during the swelling among many cell types [10].

SUMMARY

Thus, the mature differentiated lymphocytes from healthy donors in the regulation of the volume by hypotonic stress are influenced by plasmalemma surface pool reserves, the use of which depends on the elastic properties of cytoskeleton components, and the concentration of intracellular signaling molecules involved in signal transduction from receptors to cell nucleus.

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