Comparative Estimation of the "Membrane Reserve" of Blood Cells of Reptiles and Mammals

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Abstract—Based on the method of hypoosmotic loads, the value of membrane reserve and its use by blood cells of reptiles and mammals have been studied. It has been shown that lymphocytes of the both animal species have the highest reserve of plasmalemma, while frog heterophils—the lowest one. A significantly lower part of the membrane reserve participates in formation of phagosomes by mammalian neutrophils as compared with amphibian erythrocytes.

Key words: blood cells, plasma membrane, reptiles, mammals.

INTRODUCTION

The folding of the cytoplasmic membrane is a peculiarity of some cells of living organisms. The plasmalemma reserve is necessary for formation of phagosomes formed using the membrane translocation in according to the size and form of the absorbed particles and of pseudopodia providing the "ameboid" movements of pseudopodia, amebocytes in invertebrates, leucocytes in vertebrates [1]. Owing to the membrane folds, leucocytes are deformed and are going through narrow channels, the surface area increasing with the stable volume [2–4]. The regulation of the cell volume is a general biological property in different organisms—from bacteria to mammals [5]. The higher animal and human cells of renal medulla [6, 7], epithelium of lens [8] and intestine [9], cells of the nervous system [10], hepatocytes [11], erythrocytes, and leucocytes [12–15] have the capability for the volume regulation. Various systems of intracellular signalization are involved in the volume regulation [16–18]. The presence of the volume autoregulation systems indicates functional importance of this parameter [19]. Since in representatives of the class of reptiles erythrocytes are phagocytizing cells [20], while leucocytes that have a relatively small size go easily through the capillary network, the question arises about the presence and value of the "membrane reserve" of the reptile blood cells.

MATERIALS AND METHODS

The blood obtained under the ether narcosis in the animals—frog *Rana ridibunda* and laboratory white rat *Rattus norvegicus*—was used in the work. The blood of frogs was drawn from heart, while of rats—by decapitation. Heparin (10 un./ml) was used as anticoagulant. The obtained blood was centrifuged, a leucocyte-enriched lower plasma part and the leucocytic ring were collected. The erythrocyte admixture in the rat blood was destroyed using 0.83% ammonium chloride and then the leucocytes were washed out using isotonic sodium chloride solution. The object of study in the frogs was the blood fraction enriched by leucocytes by centrifugation.

To obtain data on morphological parameters, osmoregulation, osmotic resistance, and the mem-

brane reserve possibilities, a modified complex method [21] that integrates several known methods [13, 14, 22] was used. Ten μl of cell suspension was placed in each of 5 wells of the plate for microbiological studies. 100 μl of one of the sodium chloride solutions (the 1st well—isotonic, the 2nd and 3rd wells—moderately hypotonic, the 4th and 5th wells—strongly hypotonic) were added to the cells. After incubation (the 1st, 2nd, and 4th wells for 60 s, the 3rd and 5th—for 1 h), smears were prepared and stained with azur-eosin. Two cell fixation variants were used. The first—fixation in the well; 10 μl of 25% glutaraldehyde (MERCK, Germany) were added to the cell suspension. Smears were prepared from the fixed cells. The second variant—traditional: smears were prepared from the cell suspension and were fixed using glutaraldehyde or ethanol. For frogs, 0.6% NaCl solution was used as isotonic, 0.3%—as moderately hypotonic, and 0.1%—as strongly hypotonic. For rats, 0.9, 0.45, and 0.2% solutions, respectively, were used. In the stained smears, diameters of 100 cells from each pool were measured using a VideoTest system of image analysis (Microscope-Service Ltd, St. Petersburg). To determine osmotic resistance, the cell number was counted in the sodium chloride isotonic solution and after the 1-h exposition in the strongly hypotonic medium. The cell functional state was evaluated by kinetics of swelling (60 s in the moderately hypotonic solution) and from the ability to restore the initial volume by 1 h of exposition in the same medium. The membrane reserve abilities were determined from the cell size changes after the 60-s incubation in the strongly hypotonic sodium chloride solution as compared with the initial value.

The absorption ability was studied using ink particles. The 1:50 cell and ink mixture was placed into a test tube and incubated in a thermostat at 37°C for 30 min. The percent of phagocytizing cells (the phagocytic activity) and the mean number of absorbed particles (the phagocytic index) were calculated [23]. The obtained results were processed by the methods of variation statistics using special PC programs.

RESULTS AND DISCUSSION

Since during the smear preparation the cells are deformed due to their spreading on the glass, preliminary experiments were performed on the rat blood lymphocytes to find out how the morphometric parameters of cells fixed in wells and in smears are comparable. Results of the diameter measurements are presented in Table 1. The lymphocyte size differences are varied at different fixation methods from 19% (0.9% and 0.45% NaCl) to 38% (0.2% NaCl). However, in spite of differences of the absolute lymphocyte diameter values, dynamics of the cell size changes reflecting hypotonic swelling and regulatory volume restoration is practically equal. So in further studies, only the smears were used for the cell size determination.

Table 1. Diameter (μm) of blood lymphocytes incubated in iso- and hypotonic solutions at various ways of fixations

Way of fixation	NaCl concentration in solution (%), time of fixation			
	0.9	0.45, 60 s	0.45, 1 h	0.2, 60 s
In the well	5.2 ± 0.1	5.7 ± 0.1*	5.3 ± 0.1	6.0 ± 0.18
In the smear	6.2 ± 0.4	6.8 ± 0.4*	6.5 ± 0.2*	8.3 ± 0.5*

Note: M ± δ values are presented. *Asterisk* designates statistical significance of differences as compared with isotonic solution according to Student's criterion ($p < 0.05$–0.01).

The comparative assessment of the membrane reserve and cell regulatory reactions of the reptilian and mammalian blood cells has shown that lymphocytes of representatives of the both classes have an essential "reserve" of the membrane—in frog near 115%, in rat 79% (Table 2). A regulatory decrease of this cell pool volume occurs during the long-term incubation in the moderately hypotonic medium. Similar results were obtained for the rat neutrophils with the membrane reserve near 109%. The membrane reserve used by the frog erythrocytes and heterophils in the strongly hypotonic media was significantly lower: about 19% and 6%, respectively. The hypoosmotic swelling and regular shrinkage were recorded at a moderate decrease of the medium osmolarity in frog heterophils, whereas the volume variations in erythrocytes were practically absent. The osmotic resistance parameters also indicate a lower membrane reserve in frog erythrocytes (Table 3). However, the phagocytic activity and absorptive capability of frog

Table 2. Morphometric parameters of blood cells incubated in sodium chloride solutions of different osmolarity

Animal species	Kind of blood cells	Solution, time of incubation			
		IS	MH, 60 s	MH, 1 h	SH, 60 s
R. ridibunda	Lymphocytes	11.1 ± 0.6	15.5 ± 0.4*	12.1 ± 0.5*	16.2 ± 0.6*
		(384)	(754)	(460)	(824)
	Heterophils	16.9 ± 0.6	17.3 ± 0.8*	15.7 ± 0.4*	17.4 ± 0.3*
		(897)	(940)	(774)	(951)
	Erythrocytes	21.9 ± 0.5	21.6 ± 0.5	22.4 ± 0.3	23.9 ± 0.6*
		14.1 ± 0.4	14.2 ± 0.2	14.4 ± 0.3	15.5 ± 0.4
		(486)	(480)	(506)	(580)
R. norvegicus	Lymphocytes	6.2 ± 0.4	6.8 ± 0.4*	6.5 ± 0.2*	8.3 ± 0.5*
		(121)	(144)	(184)	(216)
	Neutrophils	7.6 ± 0.6	8.6 ± 0.5*	8.1 ± 0.2*	11.0 ± 0.5*
		(181)	(230)	(205)	(378)

Note: Diameter of leukocytes and the value of long (above the line) and of short (below the line) erythrocyte axes (μm) (M ± δ values) are presented; in parentheses—cell surface area (μm^2). IS—isotonic NaCl solution, MH—moderately hypotonic solution, SH—strongly hypotonic solution. *Asterisk* designates statistical significance of differences as compared with isotonic solution according to Student's criterion ($p < 0.05$–0.01).

Table 3. Osmotic resistance and absorptive ability of blood cells of different animal species

Animal species	Osmotic resistance	Phagocytosis parameters		Absorptive ability	
		phagocytic activity (%)	phagocytic index (rel. un.)	"seeming" (rel. un.)	"real" (rel. un.)
R. ridibunda	19 ± 4	100 ± 0.0	42.0 ± 3.0	8.6	7.2
R. norvegicus	69 ± 4*	75.0 ± 1.7*	7.5 ± 0.4*	4.2	2.0

Note: Absorptive ability—the relative number of particles that can be phagocytosed by cell per 100 μm^2 of plasmalemma area. *Asterisk* designates statistical significance of differences as compared with *Rana ridibunda* according to Student's criterion ($p < 0.05$).

erythrocytes turned out to be significantly higher than in rat neutrophils. The number of the absorbed particles per area unit of the cytoplasmic membrane, calculated for the cells present in the isotonic medium ("seeming" absorptive capability) was about twice higher in erythrocytes than in neutrophils. Similar calculations for the plasmalemma "flattened" due to the hypotonic swelling in the strongly hypotonic medium ("real" absorptive capability) have shown almost fourfold difference, i.e., the phagocytic activity of frog erythrocytes is higher than of mammalian neutrophils [20].

It has been shown that absorption of bacteria by frog erythrocytes occurs with formation of the membrane protrusions (pseudopodia) and subsequent formation of phagocytic vacuole. Hence, both the phagocytosed erythrocytes and mammalian neutrophils are characterized by the plasmalemma component recycling that balances absorption (endocytosis) of the membrane material [24, 25]. Results of the performed study indicate obvious "consumption" limits of the membrane reserve by mammalian neutrophils at the phagosome formation. One of explanations of this fact can be the

suggestion that the high level of metabolism in mammals is provided only under the condition of effective blood flow through the capillary network, i.e., of a relatively free passage of blood cells through microvessels. A part of the plasmalemma reserve is preserved to provide the leucocyte deformation changes. Lymphocytes of all vertebrates have similar structure [26]. In our study we have failed to reveal any differences in the membrane reserve and osmoregulation reactions of the reptilian and mammalian lymphocytes. Most likely, this cell group did not undergo essential structural and functional changes in the process of evolution.

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