THE INFLUENCE OF TEMPERATURE ON THE RELATIVE MICROVISCOSITY OF NUCLEAR RED BLOOD CELLS' MEMBRANE

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ABSTRACT

The method of the lateral diffusion of the hydrophobic pyrene probe used to determine the microviscosity of erythrocytic membrane has been shown that the increase and decrease of the temperature reduced carp's erythrocytic membrane microviscosity in comparision with that incubated at room temperature. The increase of the membrane microviscosity was detected at increasing temperature for hen and decreasing temperature for frog in comparison with room temperature. Relative membrane microviscosity of the erythrocytes depends on incubation time.

Keywords: relative microviscosity, erythrocytic membrane, carp, frog, hen.

TÓM TẮT

Ảnh hưởng của nhiệt độ đến độ vi nhớt tương đối của màng tế bào hồng cầu có nhân

Kết quả thí nghiệm bằng phương pháp khuếch tán ngang đầu dò pyren kị nước cho thấy, ở cá chép, việc tăng và giảm nhiệt độ so với nhiệt độ phòng đã làm giảm độ vi nhớt tương đối của màng tế bào hồng cầu. Độ vi nhớt tương đối của màng tế bào hồng cầu cũng tăng khi tăng nhiệt độ ở gà nhà và khi giảm nhiệt độ ở ếch so với nhiệt độ phòng. Bên cạnh đó, độ vi nhớt tương đối màng hồng cầu còn phụ thuộc vào thời gian nuôi ủ khác nhau.

Từ khóa: độ vi nhớt tương đối, màng tế bào hồng cầu, cá chép, ếch, gà nhà.

1. Introduction

The structural organization and function of biological membranes, involved in the integration of regulatory processes and reactions of the cell are focused in modern membranology [1]. Disorganization of cell membranes due to the action of various factors can lead to disruption of intracellular synthetic processes, cell maturation and release into the blood cell defective components, unable to carry out their functions [2].

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Relative microviscosity, an integral index of cell membrane integrity, plays a key role in the regulation of all processes occurring in membranes. The ralative microviscosity depends on several components: unsaturated lipids, cholesterol content, phospholipid composition and the quantity of protein that is interstitial in the membrane.This comprehensive index reflects both the structure and diffusion aspects of the lipid component of membranes and easily responds to metabolic changes and external stimuli [3-4]. As a simple and representative model we have chosen erythrocyte membrane, since its structure is quite labile and sensitive to a variety of external influences and can respond by a multitude of reversible and irreversible rearrangements in lipid and protein components [5-7]. Morphological and functional features of the membrane of red blood cells were the most detail studied in mammals and humans [8-12]. The question of the structural and functional status of the cytoplasmic membrane of nuclear red blood cells of other vertebrates is less studied.

Purpose of study: To evaluate the relative microviscosity of nuclear erythrocyte membranes under the influence of the temperature factor.

2. Materials and methods

We used peripheral blood collected from animals anesthetized with ether: hen (*Gallus domesticus*) (10 individuals), the lake frog (*Rana ridibunda* Pall.) (30 individuals), and common carp (*Cyprinus carpio*) (30 individuals). The blood samples were taken from the hen's wing large veins, the frog's heart, and the carp's caudal vein. Heparin at 10 U/mL was used as an anticoagulant. Frog and carp hemocytes were incubated for 2, 4, 6 and 8 hours at room (20°C), at reduced (5°C), and at elevated (40°C) temperatures. Sample preparation of hen blood cells was performed in similar conditions, as well as at 45°C, considering the temperatures 5°C and 20°C as reduced, 40°C - as the optimal (the body temperature of birds), and the temperature 45°C - as elevated. After incubation the blood was centrifuged for 4 minutes at 400 g, erythrocytic suspension was taken.

Estimation of the relative microviscosity of red blood cell membranes was performed using method lateral diffusion of the hydrophobic probe of pyrene ($C_{16}H_{10}$) [2]. Erythrocytic suspension was diluted with saline (0.9% for hen, 0.8% for carp, 0.6% for the frog) to absorbance 0.700 units. (in 0.5 cm cuvette at the absorption wavelength 650 nm). Incubation of the cell suspension with pyrene (Koh Light) (3 μm per 1 mL of suspension) were performed at room temperature for 1 min. under constant shaking. The ratio of fluorescence intensity of pyren excimer (F_e) and pyrene monomer (F_m) (F_e/F_m) is calculated with relation of fluorescence intensity excimers (emission wavelength – 470 nm) and monomers (emission wavelength – 395 nm). This ratio is inversely proportional to the relative microviscosity [13]. Microviscosity of the lipid bilayer of erythrocytic membranes was estimated at an excitation wavelength 334 nm,

microviscosity of areas of protein-lipid contacts – at 286 nm. Fluorescence spectra were recorded on a spectrophotometer SP-56 (Lomo Spectrum, Saint-Petersburg city).

The obtained data were processed using statistical variational methods. The average arithmetic sample (M) and the standard error of the mean (m) were calculated with computer programs Excel 7.0 and Statistica 6.0. The significance of differences between the characteristic values of the compared groups was determined using unpaired (two-sample) Student's t-test. Changes were taken at the level of statistical significance at $p < 0.05$.

3. Results and discussion

Results of ratio, characterizing the relative microviscosity of erythrocytic membranes carp are presented in table 3.1.

Table 3.1. *The ratio of fluorescence intensity of pyren excimer (F_e) and pyrene monomer (F_m), characterizing the relative microviscosity of erythrocytic membranes of Cyprinus carpio (F_e/F_m)*

Incubation temperature, °C	**F_e/F_m**	**Incubation time, hours**			
		2	**4**	**6**	**8**
5	F_e/F_m (286)	3.02 ± 0.09 &	1.73 ± 0.05 * &	1.87 ± 0.04 * # &	1.31 ± 0.03 * # @
	F_e/F_m (334)	5.01 ± 0.1 &	3.0 ± 0.09 * &	3.07 ± 0.08 * &	2.54 ± 0.06 * # @
20	F_e/F_m (286)	1.34 ± 0.02	1.38 ± 0.01	1.22 ± 0.04 * #	1.24 ± 0.03 * #
	F_e/F_m (334)	2.60 ± 0.06	2.73 ± 0.05	2.27 ± 0.03 * #	2.63 ± 0.07 @
40	F_e/F_m (286)	2.21 ± 0.06 &	1.39 ± 0.02 *	1.37 ± 0.01 * &	1.25 ± 0.02 * # @
	F_e/F_m (334)	3.70 ± 0.09 &	2.85 ± 0.05 *	2.48 ± 0.04 * # &	2.37 ± 0.05 * # &

Note: here and in tables 3.2, 3.3: F_e/F_m (286) – F_e/F_m, characterizing the microviscosity in the area of protein-lipid contacts; F_e/F_m (334) – F_e/F_m, characterizing the microviscosity of the lipid bilayer; significant difference in comparison by Student's t-criterion ($p < 0.05$) * - with the 2 hours incubation, # - with the 4 hours incubation, @ - with the 6 hours of incubation, & - with a temperature 20°C, ß - with a temperature 40°C.

Results presented in the table 3.1 showed when the temperature drops to 5°C compared to 20°C for 2-6 hours incubation F_e/F_m of the areas of protein-lipid contacts

of carp's erythrocytic membranes increased 25.36-125.37, at lipid bilayer - at 9.89-92.69 %. When the temperature rises to 40°C, the similar incubation time of erythrocytes also contributed to increase the F_e/F_m (286) and F_e/F_m (334) of carp's erythrocytic membranes to 0.72-64.93 and 4.40-42.31%, respectively, compared with the room temperature. In turn, with increasing of incubation time, studied F_e/F_m were decreased at low and high temperatures, and therefore microviscosity of annular lipid zones and lipid bilayer increases. At room temperature, with increasing of incubation time, a clear change in the dynamics of microviscosity of annular lipid zones and lipid bilayer of carp's erythrocytic membranes were not found.

Table 3.2. *The ratio of fluorescence intensity of pyren excimer (F_e) and pyrene monomer (F_m), characterizing the relative microviscosity of erythrocytic membranes of Rana ridibunda (F_e/F_m)*

Incubation temperature, °C	**F_e/F_m**	**Incubation time, hours**			
		2	**4**	**6**	**8**
5	F_e/F_m (286)	0.10 ± 0.004 &	0.45 ± 0.009 * &	0.14 ± 0.007 * # &	0.10 ± 0.003 # @ &
	F_e/F_m (334)	0.16 ± 0.007 &	1.87 ± 0.03 * &	0.81 ± 0.009 * # &	0.69 ± 0.02 * # @ &
20	F_e/F_m (286)	0.16 ± 0.005	0.13 ± 0.001 *	0.17 ± 0.006 #	0.44 ± 0.01 * # @
	F_e/F_m (334)	0.55 ± 0.02	0.29 ± 0.008 *	0.92 ± 0.009 * #	1.73 ± 0.02 * # @
40	F_e/F_m (286)	0.08 ± 0.005 &	0.15 ± 0.004 * &	0.50 ± 0.003 * # &	0.34 ± 0.04 * # @ &
	F_e/F_m (334)	0.60 ± 0.006 &	0.98 ± 0.03 * &	1.98 ± 0.02 * # &	1.03 ± 0.05 * @ &

The table showed when the incubation temperature reduced to 5°C in 2, 6 and 8 hours, F_e/F_m (286) of frog's erythrocytic membranes was 37.50, 17.65 and 77.27% (F_e/F_m (334) was 70.91, 11.96 and 60.12%) down from the temperature 20°C, respectively. In 4 hours incubation, at a low temperature, studied ratios, characterizing microviscosity of frog's erythrocytic membranes in area of annular lipids and in a lipid bilayer were more twice and 5 times higher than at room temperature, respectively. When the temperature rises to 40°C, 2-hour incubation led to reduce F_e/F_m (286) by 50.0% and increase F_e/F_m (334) – by 9.09% compared with a room temperature. A

marked increase in temperature has also led to an increase in F_e/F_m ratios, characterizing the microviscosity of frog's erythrocytic membrane in the area of protein-lipid contacts by 15.38 and 194.12% in 4 and 6 hours of incubation, respectively. Under similar conditions of sample preparation, studied ratios in the lipid bilayer increased more twice. In 8-hour incubation, F_e/F_m (286) and F_e/F_m (334) at 40°C were 22.73% and 40.46%, repestively, down from the room temperature. With increasing incubation time, ratios, are inversely proportional to the relative microviscosity of the lipid phase and annular lipid areas of frog's erythrocytic membranes both at low and at high temperatures, increased, and the maximum values were recorded at 4- and 6-hour incubation. At room temperature, increasing the incubation time detected a similar decline of the relative microviscosity of frog's erythrocytic membranes, except indicators recorded in 4-hour incubation.

Indices of the relative microviscosity of hen's erythrocytic membrane are shown in table 3.3.

Table 3.3. *The ratio of fluorescence intensity of pyren excimer (F_e) and pyrene monomer (F_m), characterizing the relative microviscosity of erythrocytic membranes of Gallus domesticus (F_e/F_m)*

Incubation temperature, °C	**F_e/F_m**	**Incubation time, hours**			
		2	**4**	**6**	**8**
5	F_e/F_m (286)	2.98 ± 0.08 ß	2.32 ± 0.07 * ß	4.57 ± 0.09 * # ß	2.87 ± 0.06 # @
	F_e/F_m (334)	3.86 ± 0.06 ß	4.99 ± 0.1 * ß	7.65 ± 0.18 * # ß	5.78 ± 0.09 * # @
20	F_e/F_m (286)	2,82 ± 0.04 ß	2.67 ± 0.05 * ß	4.50 ± 0.09 * # ß	1.89 ± 0.02 * # @ ß
	F_e/F_m (334)	6.17 ± 0.13 ß	5.74 ± 0.1 *	7.42 ± 0.16 * # ß	4.49 ± 0.06 * # @ ß
40	F_e/F_m (286)	2.17 ± 0.03	2.88 ± 0.02 *	3.03 ± 0.05 * #	2.79 ± 0.03 * # @
	F_e/F_m (334)	4.94 ± 0.13	5.62 ± 0,07 *	5.86 ± 0.14 *	5.65 ± 0.1 *
45	F_e/F_m (286)	1.31 ± 0.02 ß	1.31 ± 0.01 ß	1.40 ± 0.009 * # ß	1.09 ± 0.008 * # @ ß
	F_e/F_m (334)	3.56 ± 0.04 ß	3.52 ± 0.03 ß	4.44 ± 0.05 * # ß	3.16 ± 0.04 * # @ ß

The table 3.3 showed that lowering the temperature to 5°C compared to 40°C contributed to the increase of F_e/F_m (286) hen's erythrocytic membrane by 37.33 and 50.83% in 2 and 6 hours and contributed to the decline by 19.44% in 4 hours incubation. In similar decrease of the incubation temperature, the F_e/F_m (334) of hen's erythrocytic membranes in 2 and 4 hours has reduced by 21.86 and 11.21%, in 6 hours was up 30.55%, respectively. By lowering the incubation temperature to 20°C compared to 40°C, F_e/F_m (286) of hen's erythrocytic membranes in 2 and 6 hour increased by 29.95 and 48.51%; in 4 and 8 hours – decreased by 7.29 and 32.26%, respectively. Under above-mentioned conditions, F_e/F_m (334) of hen's erythrocytic

membranes in 2 and 6 hours increased by 24.90 and 26.62%, in 8 hours – decreased by 20.53%. Increasing the incubation temperature to 45°C for 2-8 hours led to the decrease of the ratio, characterizing membrane microviscosity of the zones of protein-lipid contacts, by 39.63 – 60.93% (the ratio, characterizing microviscosity in the lipid bilayer has decreased by 24.23 – 44.07%) compared to 40°C. In hen, the lowest values of membrane microviscosity were detected at 6 hours incubation, and the higher the temperature, the smaller the difference with the indicator, obtained by a 4 hour incubation.

Increasing the relative microviscosity in the areas of protein-lipid contacts and of lipid bilayer of experimental animal's erythrocytic membranes indicates a violation of stability, reducing fluidity [10], change in the viscoelastic properties of the membrane and increasing the cell rigidity. In the clinic, this state is called "hardness" of red blood cells, which means a decrease in their deformability or its deprivation. In addition to the loss advance ability of red blood cells in the microvasculature, their osmotic resistance has been decreased and hemolysis has been increased [2]. Increasing the incubation time both at elevated and at low temperatures reduces the microviscosity of frog erythrocytic membrane both in the area of annular lipid and in lipid bilayer, that indicates the normalization of lipid-lipid and protein-lipid interactions in the membrane [1]. Hen's erythrocytes have the lowest values of membrane microviscosity at 6-hour incubation, regardless of the temperature. According to the literature, decreasing the membrane microviscosity contributed to rising of unsaturated lipids (increase in the number of double bonds). It is particularly important is the appearance of the first double bond in the lipid molecule, and with increasing the degree of unsaturation, the effect decreases progressively [14, 15].

4. Conclusion

In carps, increase and decrease of the incubation temperatures compared to room temparture have reduced the microviscosity carp erythrocytic membrane. When the incubation time was risen, the relative microviscosity of the erythrocytic membranes has increased both at low and at high temperatures.

The microviscosity of the erythrocytic membranes was increased at high incubation temperature (in *Gallus domesticus*) and at low (in *Rana ridibunda*) incubation temperatures, compared to the room temperature.

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