Journal of Stress Physiology & Biochemistry, Vol. 12 No. 3 2016, pp. 5-11 ISSN 1997-0838

ORIGINAL ARTICLE

Functional State of Rat's Erythrocytes Under Different Stress Conditions

A.A. Martusevich[1], A.V. Deryugina[1], A.K. Martusevich[2]

[1] *Department of physiology and biochemistry of the human and animals, National Research Nizhny Novgorod State University named after N.I. Lobachevsky, 603000, Nizhny Novgorod, Gagarin st., 23, Russia.*
[2] *Experimental medicine department, Privolzhsky Federal Medical Research Centre, 603155, Nizhny Novgorod, Verhnevolzhskaya emb., 18, Russia.*

*E-Mail: *cryst-mart@yandex.ru*

Received May 24, 2016

Background: In our early publications was shown that electrophorhetic motility of erythrocytes (EPME) is a high effective criteria of adaptation response. This correlation is based on parallel development of adaptation syndrome and activation of the main organism regulatory systems, such as sympatoadrenalic and hypotalamo-hypophosial-adrenal ones.

Objective*:* study of the influence of physical exercises and adrenaline injections on electrophorhetic motility, membrahes phospholipids spectrum and oxidative metabolism of the rats' erythrocytes.

Methods*:* Rats were divided into three equal groups. First group of animals was control (n=10; without any manipulations). Rats of second group were subjected to physical load in the form of a sailing duration of 15 minutes with a cargo amounting to 10% of animal body weight (water temperature – 26-28^{0}C). Rats of third group were intraperitoneally injected with adrenaline hydrochloride (0.1 mg/kg). Blood sampling was made from the sublingual vein in 15, 30, 60, 120 minutes and 24 hours after exposure. We estimated the dynamics of the electrophorhetic motility of erythrocytes (EPME), the phospholipid spectrum of erythrocytes membranes, the concentration of malonic dialdehyde (MDA) and the state of the glutathione system.

Results and conclusions*:* The study suggests that red blood cell as a biological system is capable for realization of stress response may develop a special "alarm reaction" after action of the stress agent. This response initiates activation of free radical processes and phospholipids profile in erythrocyte membranes with reducing of its electronegativity. This stage enhances the activity of the antioxidant system, is limiting the development of lipid peroxidation processes, and leads to the development of "adaptation stage" of the cellular system, coupled with the restoration of the electronegativity of the membrane and the mobilization of reserves of low molecular antioxidants, particularly glutathione, as well as "structural antioxidant", due to the content of readily oxidizable lipid (phosphoethanolamine) and lipids that are resistant to oxidation (phosphatidylcholine, sphingomyeline).

Key words: blood, glutathione, malonic dialdehyde, stress factors, phospholipids

It is well-known that organism response on any negative influences is associated with stereotype complex of different molecular, cellular and tissue transformations, which are including in the term "adaptation syndrome" (Maslova, 2005). That is why study of general laws of adaptation processes has a constant relevance is causing by necessary of improvement of its theoretic basis and methods of targeted correction and prophylaxis (Matteuci E. *et al*., 1992; Krylov *et al*., 1998).

Crucial role in implementation of adaptation plays the different components of blood, first of all erythrocytes, participating in homeostasis keeping in whole organism (Krylov *et al*., 1998). In our early publications was shown that electrophorhetic motility of erythrocytes (EPME) is a high effective criteria of adaptation response (Krylov, Derugina, 2005). This correlation is based on parallel development of adaptation syndrome and activation of the main organism regulatory systems, such as sympatoadrenalic and hypotalamo-hypophosial-adrenal ones (Krylov, Derugina, 2005; Maslova, 2005). Given that the EPME variations are mediated by reorganization of the physical and chemical structure of the erythrocyte membrane, it is necessary to assume the existence of a universal stereotype of changes of the structural and functional state of erythrocytes and its environment in response to the action of stress factors.

That's why the aim of this work is study of the influence of physical exercises and adrenaline injections on electrophorhetic motility, membranes phospholipids spectrum and oxidative metabolism of the rats' erythrocytes.

MATERIALS AND METHODS

The study was conducted on 30 white Wistar rats weighing 200-250 g. The animals conformed to the rules for the design, equipment and maintenance of experimental biological clinics (vivarium). Animals were fed natural feed in accordance with regulations. Animals were quarantined and acclimatized under conditions of vivarium within 14 days.

Rats were divided into three equal groups. First group of animals was control (n=10; without any manipulations). Rats of second group were subjected to physical load in the form of a sailing duration of 15 minutes with a cargo amounting to 10% of animal body weight (water temperature – 26-28^{0}C). Rats of third group were intraperitoneally injected with adrenaline hydrochloride (0,1 mg/kg).

Blood sampling was made from the sublingual vein in 15, 30, 60, 120 minutes and 24 hours after exposure. We estimated the dynamics of the EPME, the phospholipid spectrum of erythrocytes membranes, the concentration of malonic dialdehyde (MDA) and the state of the glutathione system. Erythrocytes is using in our experiments was washed three times with a 0,85% solution of sodium chloride and centrifuged during 10 min at 1500 rpm. Measurement EPME was performed by the method of micro-electrophoresis (Kharamonenko, Rakityanskaya, 1974), registering the passage of red blood cells a distance of 10 μm in Tris-HCl buffer with pH 7.4 with an electric current was 10 mA. The MDA concentration was determined photometrically by the reaction with thiobarbituric acid. For the characterization of the glutathione system we evaluated the concentration

of total, oxidized and reduced glutathione (Sedlak, Lindsey, 1968). The separation of the lipid components of erythrocyte membranes was performed by the method of one-dimensional thin-layer chromatography (Sharshunova *et al.*, 1980).

Statistical analysis of the data was performed with Statistica 6.0 program. Data were expressed as means ⑥ SE, the Student's t-test was used for detection of statistical difference.

RESULTS

Our experiments shown that dynamics of EPME has a stereotype character as under physical exercises, as at intraperiteneal injection of adrenaline. Both of these condition associate with primary decreasing of the parameters with its subsequent normalization. In addition, the severity of EPME changes depended on the action strength.

Analysis of phospholipid composition of erythrocyte membranes showed similar direction of change of the content of phospholipid fractions in the studied alterations, which includes the increase in the lysoforms (LF) concentration and a decrease in sphingomyelin (SM) level. After the adrenaline administration we also observed a growth in the concentration of phosphatidylcholine and a decrease in the level of phosphatidylethanolamine. This tendency may indicate a more powerful effect of adrenaline on the body than swimming. This notion is confirmed by stronger changes of LF and SM level compared with physical activity. By the end of the first day of the experiment we fixed the partial restoration of the phospholipid spectrum of erythrocyte membranes in adrenal toxemia and full recovery during physical activity (table 1).

These results coincide with the results obtained previously in the study of EPME and phospholipid spectrum of erythrocyte membranes at various kinds of diseases (Krylov *et al.*, 1998; Krylov, Derugina, 2005). Given that the basis of the pathological process is a different severity of stress reactions, it can be argued that stereotype EPME changes is associating with same dynamics of phospholipids content of erythrocyte membranes reflect a systemic response on influence of the stress factors, including an imbalance of pro - and antioxidant systems.

Estimation of pro- and anyioxidant systems state in rats' blood revealed the similarity of the registered changes under the used stress conditions, but its maximal dynamics was fixed under the adrenaline administration.

Study of lipid peroxidation (LPO), is traditionally measuring by the formation of MDA (Matteuci E. *et al.*, 1992), showed that level of this substance elevates in 15 minutes after the action of stress factors. At other side the restoration time of MDA concentration depends on the factor type. In particular, we observed normalization of MDA level in one hour after the swimming, but in adrenal toxemia this period was 1 day (table 2).

Given that an important component of antioxidant protection of erythrocytes is the glutathione system, which is the buffer system that protects red blood cells from the destructive action of oxidizing agents, a study was conducted on the state of the glutathione system. In animals subjected to short-term physical activity has recorded a relatively weak increase in the concentration of total glutathione in erythrocytes, with a maximum of 60 minutes of observation and followed reduction of the level

of the parameter to its original value. We fixed increasing of the total glutathione level from 15 min after the adrenaline administration. Parameter was elevated during all the experiment. It should be noted that the change in the total glutathione was observed due to the growth of its restored form (table 2).

These results showed that the unidirectional structural and functional reorganization of erythrocyte membranes as a response to various stress factors is associated with adaptive rearrangements of cell-mediated development of adaptation processes.

Table 1. Dynamics of erythrocyte electrophorhetic motility and lipid profile of erythrocyte membranes under different types of the stress

Parameter	Time after the exposure	Intact rats	Type of the stress	
			Swimming	Adrenaline injection
Erythrocyte electrophorhetic motility, mcm*sm*V/s	15 min	1,02±0,02	0,97±0,06	0,68±0,09*
	30 min	1,00±0,01	0,93±0,04*	0,69±0,08*
	60 min	1,04±0,02	1,07±0,02	0,72±0,08*
	120 min	1,05±0,02	1,1±0,04	0,63±0,07*
	1 day	1,02±0,04	1,06±0,07	0,83±0,09*
Lysoforms, %	15 min	4,13±0,20	4,2±0,25	9,33±0,37*
	30 min	3,98±0,29	4,0±0,2	12,05±0,46*
	60 min	5,5±0,2	5,42±0,29	13,28±0,44*
	120 min	4,25±0,21	5,04±0,32*	14,73±0,58*
	1 day	3,57±0,23	3,71±0,21	10,43±0,30*
Phosphatidyl-choline, %	15 min	34,56±1,91	36,22±1,53	46,37±0,41*
	30 min	34,92±1,62	36,26±1,48	45,72±1,57*
	60 min	40,3±0,73	40,66±1,21	45,85±0,81*
	120 min	38,72±1,53	40,52±1,18	49,0±1,54*
	1 day	39,4±0,40	41,23±0,96	44,86±1,53*
Phosphatidyl-ethanolamine, %	15 min	37,67±2,13	35,55±1,88	25,93±0,20*
	30 min	38,35±1,12	36,24±1,66	25,10±1,75*
	60 min	30,2±0,49	31,3±0,96	24,62±0,94*
	120 min	32,9±1,76	32,4±1,08	20,67±1,28*
	1 day	31,63±0,59	32,65±0,87	26,87±1,01*
Sphingomyeline, %	15 min	23,63±1,17	23±0,74	18,37±0,47*
	30 min	22,75±0,83	22,1±1,05	17,02±0,50*
	60 min	24,0±1,16	22,62±1,3	16,25±0,66*
	120 min	24,12±0,48	22,04±1,31*	15,6±0,81*
	1 day	24,2±0,52	22,41±1,15	17,83±0,37*

Table 2. Dynamics of malonic dialdehyde level and state of glutathione system in erythrocyte under different types of the stress

Parameter	Time after the exposure	Intact rats	Type of the stress	
			Swimming	Adrenaline injection
malonic dialdehyde, mmol/ml	15 min	3,06±0,10	3,84±0,08*	3,97±0,10*
	30 min	3,99±0,12	4,46±0,09*	5,41±0,16*
	60 min	4,73±0,08	4,42±0,14	6,06±0,08*
	120 min	5,06±0,13	5,04±0,12	6,43±0,08*
	1 day	3,17±0,09	3,31±0,10	4,03±0,18*
Restored glutathione, mg%	15 min	70,9±1,90	86,63±1,83*	153,0±2,82*
	30 min	72,12±4,27	104,23±2,50*	166,13±6,14*
	60 min	74,12±2,56	105,27±2,46*	157,2±5,51*
	120 min	74,1±2,17	101,68±4,96*	163,33±4,7*
	1 day	70,88±3,66	79,65±2,48	161,03±3,12*
Oxidated glutathione, mg%	15 min	15,73±3,16	13,05±1,82	23,38±3,16
	30 min	18,73±2,23	21,98±2,17	24,78±2,91
	60 min	27,07±3,31	31,72±3,32	22,43±2,71
	120 min	23,05±1,72	22,48±3,21	25,28±2,88
	1 day	15,35±3,14	22,48±3,2	17,25±1,91
Total glutathione, mg%	15 min	86,63±3,36	99,68±3,12*	176,38±3,12
	30 min	91,5±4,91	126,22±2,68*	190,92±6,37*
	60 min	101,48±3,45	136,98±5,91*	179,63±5,71*
	120 min	97,62±2,79	124,17±4,74*	188,62±5,60*
	1 day	86,23±3,68	85,14±3,05	161,03±3,12*

DISCUSSION

The similarity of EPME changes (decreasing of the parameter) under various extreme conditions and the pathology is a common non-specific reaction determined by the development of stress response. EPME level is closely related with phospholipid spectrum of red blood cells that is associated with activation of sympatho-adrenal system. The first reaction of the blood system to stress, is accompanied by the appearance of digoxin-like factor in peripheral blood[1]. This substance inhibits the activity of membrane Na^+,K^+-ATPase, that resulting to the accumulation of Ca^{2+} in the cytosol of the cell.

The increase in Ca^{2+} level leads to activation of phospholipases, increasing of lisoforms level (Korotaeva *et al.*, 1997) and inhibition of the sphingomyelase activity, which causing the reduction in the proportion of sphingomyelin. In turn, change of EPME and phospholipids spectrum reflect typical realignment of erythrocyte membranes and is mediated by activation of LPO processes and changes in the antioxidant status of the organism. In particular, decreasing of phosphoethanolamine is known as a readily oxidizable fraction and main LPO substrate is associated with activation f this process.

Membranes micro-mechanics is also depends to present LPO level (Maslova, 2005). This influence realizes in significant reducing of the of liquid lipids amount in the bilayer, as well as the level of immobilized membrane lipids. At that time the share of close-packed lipids in bilayer increases dramatically (Krylov *et al.*, 1998). Depletion of membrane phospholipids with unsaturated acyl chains, is leading to its denser packaging, increases the viscosity of the lipid bilayer due to lipid peroxidation. The formation of lipid peroxides in the membrane is accompanied by an increase of its permeability (Kozinets, 2007). The increase in passive kationic permeability of the membranes is accompanied by leakage out of cells and potassium ions with decreasing of EPME (Kozinets, 2007). Intensification of antioxidant processes in cells, is keeping the generation of oxidative stress, restores phospholipid spectrum and the electronegativity of the cell membranes. However, given that the pentose phosphate cycle activates during the recovery of oxidized glutathione, we can assume the rearrangement of cell metabolism in this case.

Thus, the different stress agents, such as a swim test and the adrenaline injections, cause the universal response of the cellular system associated with the development of the stress reaction. On the cellular level it realizes as a series of adaptation processes, which can initiate the formation of general adaptation syndrome (its activation and resistance stages). Stage of activation, which is manifested in the reduction of EPME and activation of sympatho-adrenal system (Krylov, Derugina, 2005), accompanied by a initiation of LP and changing of phospholipids composition in the membranes. Stage of resistance, determined by the action of glucocorticoids, and characterized by EPME growth (Kozinets, 20), is connected with increase in the concentration of total and reduced glutathione, reducing the MDA concentration and restoring phospholipid status of the membranes.

The study suggests that red blood cell as a biological system is capable for realization of stress response may develop a special "alarm reaction" after action of the stress agent. This response initiates activation of free radical processes and phospholipids profile in erythrocyte membranes with reducing of its electronegativity. This stage enhances the activity of the antioxidant system, is limiting the development of lipid peroxidation processes, and leads to the development of "adaptation stage" of the cellular system, coupled with the restoration of the electronegativity of the membrane and the mobilization of reserves of low molecular antioxidants, particularly glutathione, as well as "structural antioxidant", due to the content of readily oxidizable lipid (phosphoethanolamine) and lipids that are resistant to oxidation (phosphatidylcholine, sphingomyeline).

It should be noted that EPME level can be an additional parameter, which characterize the blood response on different types the stress, like a present LPO status, antioxidant activity and changes of the membranes phospholipids profile. In whole, EPME subtly reacts to the changing state of the organism even in itself, but in combination with other physical and chemical parameters of the blood for full estimation of morphofunctional pattern of each stages of the adaptation process.

REFERENCES

Kharamonenko S.S., Rakityanskaya A.A. (1974)

Electrophorhesis of blood cells in physiology and pathology. Minsk: Belarus.

Korotaeva A.A., Cheglakov I.B., Morozkin A.D., Suslova I.V., Prokazova N.V., (1997) Effect of lysophosphatidylcholine on the structure and function of low density lipoproteins. *Membr. Cell Biol.* **10(5)**. 521-534.

Kozinets G.I., Popova O.V., Budnik M.I. (2007) The electrical charge of the blood cells: laboratory and clinical value. Moscow.

Krylov V.N., Derugina A.V. (2005) Stereotype changes of electrophorhetic motility of the erythrocytes under the stress. *Bulletin of Experimental Biology and Medicine.* **139(4)**. 364-366.

Krylov V.N., Gustov A.V., Derugina A.V. (1998) Electrophorhetic motility of the erythrocytes and stress. *Human physiology.* **24 (6)**. 108-111.

Maslova M.N. (2005) Molecular mechanisms of the stress. *Russian physiological journal*. **91(11)**. 1320-1328.

Matteuci E., Cocei F., Pellegrini L. (1992) Erythrocyte ATP-ase enzymes family in normal people. *Eur. J. Clin. Invest.* **4.** 11.

Sedlak J., Lindsey R.H. (1968) Estimation of total protein bound, non-protein sulfhydryl groups in tissue with Ellmans reagent. *Anal. Biochem.* **2**. 192-205.

Sharshunova M.V., Schvarts V.I., Mikhalets Ch.G. (1980) Thin-layer chromatography in pharmacy and clinical biochemistry. Moscow, 536 p.