Current Topics in Biophysics 2001, 25(1), 27-31

THE INFLUENCE OF A NITROGEN OXIDE ON ERYTHROCYTE DEFORMABILITY IN RATS EXPOSED TO STRESS OF WATER IMMERSION

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The influence of a modification of nitrogen oxide synthase activity on erythrocyte deformability in rats during the stress caused by water immersion have been investigated. The obtained results show that a blockage of a nitrogen oxide synthase during water immersion leads to a relatively considerable decrease of erythrocytes deformability, whereas an increase of nitrogen oxide activity causes a moderate increase in their deformability.

INTRODUCTION

Stress in a sign of a whole range of changes through which an organism responds to the agents have are capable to evoke specified inner reactions, such as an intensive nervous tension, anxiety reaction, excitation increase, etc. This is also a phenomenon occurring in surgery. An illness itself, as well as an immobilization of a sick person awaiting an operation, and, finally damaging agents like: an operation, or a possible infection are also stress-evoking factors. Among various changes connected with stress, some essential changes of rheological blood agents properties, such as a decrease of erythrocytes deformability and increase of their aggregation and vessel endothelium adhesion may also occur (Chang, Absolom, Strong, Stubley & Zingg, 1988; Mohandas & Chasis, 1993). Erythrocytes deformability matters on the level of arterioles as well as capillary vessels. In arterioles, in which a velocity gradient is high, erythrocytes deformability occurs and their rigidity is lower. Deformed erythrocytes show a lower hydrodynamic impact on a dispersed phase, and, as a result, blood fluidity increases. In the case of a flow of erythrocytes which have a lower deformability, hydrogen impacts increase and blood fluidity decreases (Chien, 1987; Gaetgens, Duhrssen & Albrecht, 1980; Stuart & Nash, 1990). However, on the level of capillary vessels whose diameter is considerably smaller than this of erythrocytes, a flow of erythrocytes is possible only when its diameter is smaller. It is possible when blood cells are elastic. A blood cells flow through nutrient vessels impossible when blood cells are rigid. It leads to the disorders in cell and tissue blood supply (Maeda & Shiga, 1994; Sarelius, 1995; Shiga, 1994). A reduced erythrocytes deformability probably occurs also in stress. For example, it has been experimentally ascertained that stress reaction occur in a process of an anaesthetising rabbits with a mixture of halotan and pentabarbital and were accompanied by a transitory decrease of erythrocytes deformability (Yesilkaya, 1998).

One of the experimental stress models is animals water immersion. It leads to a activation of sympathetic system and blood flow disorders of microcirculation (Reilly, Rivalier, Compagnon & Laplane, 1934). Some authors were observing an occurrence of blood flow disorders in capillary vessels as a result of heavy exercise, which was associated with a decreased erythrocytes deformability (Van der Brug, 1995). Among others, a gastric mucosa damage, in a form of ulcers, occurs to tested animals when subjected to water immersion. It is assumed that these damages are connected with an excessive nitrogen oxide production, which can be proved by the fact that changes in gastric mucosa could be removed by using a nitrogen oxide synthase inhibitor (Nishida, Ohta & Ishiguro, 1998).

Up to the present, a nitrogen oxide influence on erythrocytes deformability has not been established explicitly. It is assumed that both too little and too much of the agent's concentration causes erythrocytes rigidity (Korbut & Gryglewski, 1993). Particularly, it concerns animals subjected

to an oxidation stress which considerably sentisizes erythrocytes to the agents decreasing erythrocytes elasticity through a peroxidase.

The aim of this work was to estimate the influence of a nitro oxide synthase activity modification on erythrocytes deformability during the stress evoked by water immersion of tested animals.

MATERIAL AND METHODS

The research material consisted of thirty rats-males of Spraque Dawley breed weighing from 250 to 300g. Rats were divided into two groups of 15 individuals each, chosen at random. One of the groups was a control group A, the other onosubjected to water immersion – was group B. Three subgroups were randomly separated from each group, and each subgroup consisted of 5 individuals.

Subgroups A-1 and B-1 were only administrated 2 ml of 0,9% NaCl solution intraperitoneally, 2 ml of NaCl solution and 10 mg/kg of body weight of nitrogen oxide synthase inhibitor in a form of Nw-nitro L-arginine methylester (L-Name) was administrated to subgroups A-2 and B-2, whereas l-Arginina – 25 mg/kg of body weight – was administrated to subgroups A-3 and B-3. Afterwards, rats from group B were took subjected to the stress of water immersion. The procedure took a following course: a rat was immersed into the water of 23^{o}C, at its xiphoid process level, for four hours, in a vertical position; then it was anaesthetized with ether. After dissecting of the sternum, the blood was collected out of the cardiac ventricle. Using Rheodyn SSD-Myrenne apparatus (Roengen-Germany), a measurement of erythrocytes elongation index was carried out.

The way the apparatus works is based and automatic analysis of diffraction images, which were obtained by means of a laser ray going through erythrocytes suspension in a suitable dextran solution. A blood cells deformation occurred as a result of growing coagulating tensions generated between coaxial plates. Coagulating tensions expressed in [Pa]: 0.3; 0.6; 1.2; 3.0; 6.0; 12.0; 30.0; 60.0 were employed. A measuring procedure was controlled by a proper computer software.

An elongation index has been calculated the basis of light intensity of a foreground part of erythrocyte picture diffraction spectrum in both orthogonal axes which were distanced equally from the center of the picture, according to the formula:

Table. 1 The statistical analysis of erythrocyte elongation index in subgroup of control group ($\overline{X} \pm S_D$, and varation index [%]).

Shear stress Pa	Elongation index[%]		
	Subgroups		
	A-1	A-2	A-3
0.3	12.03 ± 0.7 5.8	11.53 ± 0.9 7.8	12.57 ± 1.2 9.5
0.6	16.56 ± 2.0 12.0	16.16 ± 0.9 5.5	16.93 ± 1.5 8.8
1.2	21.35 ± 2.0 9.3	20.94 ± 0.8 4.0	21.52 ± 1.3 6.0
3.0	29.18 ± 1.5 5.0	28.93 ± 0.7 2.4	28.97 ± 1.1 3.8
6.0	35.1 ± 0.9 2.6	35.1 ± 0.6 1.7	34.5 ± 0.5 1.4
12.0	39.5 ± 0.8 2.0	39.9 ± 0.7 1.7	38.3 ± 0.6 1.5
30.0	42.9 ± 1.2 2.8	43.5 ± 1.0 2.3	40.9 ± 1.3 3.2
60.0	43.2 ± 1.2 2.8	43.8 ± 1.0 2.3	41.2 ± 1.3 3.1

Lack of statistical significance of erythrocyte elongation index in subgroups of control group.

$$IE = \frac{L - W}{L + W} 100\% ,$$

where, L – length of ellipsis, W – width.

Each rat 5 IE measurements taken in five different samples of the same blood. An arithmetical mean was the result of the measurement. A margin of error a single measuring did not exceed 0.5%. The same examination was performed in the control group. Its importance has been verified by means of variance analysis.

RESULTS

Average values of *IE*, a standard deviation, and a variability index in control subgroups a function of coagulating tension have been compared in Table 1.

As we can see in presented data, no important deformability differences between subgroups of the same group have occurred in used coagulating tensions.

The results research in group B have been compared Table 2.

The erythrocytes *IE* of a B-1 subgroup was not much different from the one of a subgroup A-1. In comparison with A-1 subgroup there occurred some tendencies of *IE* decreasing in a subgroup B-1 to coagulating velocity from 3 Pa to 30 Pa. An average, relative *IE* drop in the number in this range did not exceed 2.3%; no differences were recorded at coagulating tensions of 60 Pa. It indicates that water immersion stress used caused only a slight increase of erythrocytes *IE*.

Some essential changes of the *IE* were caused by adding substances modifying the activity of a nitrogen oxide synthase along with the stress evoked by water immersion. An essential decrease of erythrocytes *IE* occurred in relation to both subgroups A-1 and B-1, however, there occurred an essential increase in erythrocytes *IE* parameter in a subgroup B-3, both in relation to subgroups A-1 and B-2. The relative changes of IE in examined subgroups depended on a coagulating tension. These changes in the subgroups of the examined group in relation to the subgroup A-1 have been presented in Fig. 1.

A relative erythrocytes IE decrease in the subgroup B-2 in comparison with the subgroup A-1 was the highest at tension of 0.3 Pa, however at 60 Pa it was 9.2%. Similar changes occurred in comparison with the subgroup B-1. In the subgroup B-3 the increase of the IE occurred, and its

Table 2. The statistical analysis of erythrocyte elongation in subgroups of examined group ($\overline{X} \pm S_D$. and veriation index [%]).

Shear stress Pa	Elongation index [%]		
	Subgroups		
	B-1	B-2	B-3
0.3	12.30 ± 1.44	10.59 ± 0.97 *	15.22 ± 1.02 ***
	11.7	9.1	6.7
0.6	17.10 ± 1.60	14.14 ± 0.51 ***	18.83 ± 0.51 *
	9.3	3.6	2.7
1.2	21.52 ± 1.56	19.01 ± 1.20 **	23.72 ± 0.34 **
	7.2	6.3	1.4
3.0	28.75 ± 1.42	26.12 ± 1.13 ***	30.96 ± 0.43 ***
	4.9	4.3	1.4
6.0	34.43 ± 1.26	31.89 ± 1.64 ****	36.79 ± 0.88 ****
	3.6	5.1	2.4
12.0	38.60 ± 1.15	36.26 ± 2.68 **	41.07 ± 1.56 ***
	2.9	7.4	3.8
30.0	41.44 ± 1.12	39.29 ± 2.11 *	44.04 ± 2.28 *
	2.7	5.3	5.1
60.0	43.23 ± 1.19	39.55 ± 2.22 *	44.33 ± 2.33 *
	2.7	5.6	5.2

* p< 0.05 ** p< 0.02 *** p< 0.01 **** p<0.002

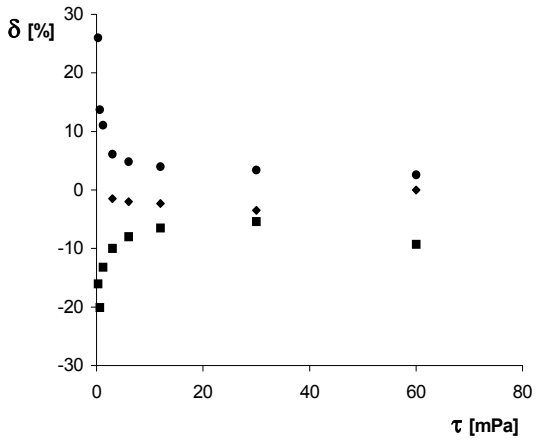

Fig. 1. The relative change of erythrocyte elongation index versus subgroup A-1: ◆ - in subgroup B-1, ■ - in subgroup B – 2 , ● - in subgroup B – 3.

increase was between ab. 25% to ab. 10% at coagulating tensions from 0.3 to 1.2 Pa; however, at higher coagulating tensions the increase was lower and amounted adequately 6-2.5% at coagulating velocity of 3.0-60.0 Pa.

DISCUSSION

The results of our research indicate that in comparison to the subgroup A-1 there has only occurred a slight *IE* decrease in the group of rats subjected to stress by means of water immersion (subgroup B-1). The decrease has only occurred at the coagulating tensions ranging from 3.0 to 30.0 Pa. Therefore, the erythrocytes deformability decrease in the present experiment was lower in comparison with the previous research (Chmiel, Grabowska-Bochenek & Piskorska, 2001), in which the duration of water immersion stress activity was 6 hours. It is probable that stress lasting 4 hours was too short to result in considerable erythrocytes deformability decrease. The IE decrease that happens under the stress is connected with generating products which are of a very high reactivity, such as reactive oxygen forms, hydrogen, hydroxyl radicals, oxygen radicals and nitrogen oxides (Baskurt, Temiz & Meiselman, 1998). It has been ascertained that these products are the cause of many processes demaging cells and tissues (Soczyński & Bartosz, 1996; Stour, Bilto & Juma, 2000). The oxidation of superficial cells components is of great importance in these processes and, especially, the oxidation of cell membrane lipids, proteins and proteglicans degradiation (Beckman, Beckman, Chen, Marshall & Freeman, 1990). The changes, which have been described above, also concern erythrocytes (Soczyński & Bartosz, 1997), and in consequence a deformability decreases. A merely slight IE decrease, shown in our research in the subgroup B-1 has its explanation in a relatively short time of animal exposition to stress. A decrease in erythrocytes deformability, evoked by an oxidational stress, through both intracellular and extracellular generation of a superoxide anion-radical has been observed by Baskurt and his co-workers (Baskurt *et al.*, 1998).

The nitrogen oxide activity modification, which we used in rats control group did not cause considerable alterations in erythrocytes deformability. It seems contradictory to research results of other authors (Starzyk, Korbut & Gryglewski, 1999) who were observing a decrease of erythrocytes deformability after administrating L-Name to the tested animals. However they used shorter time of observation and a different way of medicine administration, which may justify the observed divergence of results.

Whereas in the circumstances of stress evoked by water immersion (subgroup B-2), a nitrogen oxide synthase blockage caused in our research a considersable decrease of erythrocytes deformability. These results are consistent with the results of other authors (Zinchuk & Borisiuk, 1998), who while investigating the impact of a simultaneous application of L-Name and a hyperthermia on rats, also found decrease in erythrocytes deformability. In the course of our research we stated that the intensification of a nitrogen oxide synthase activity improves erythrocytes deformability (subgroup B-3), Other authors who had found the erythrocytes deformability increase after an intravenous administration of nitrogen oxide donor (SIN-1) came to congruent results (Starzyk *et al.*, 1999). On the basis of the obtained results, a hypothesis arises that the stress evoked by water immersion sensibilizes erythrocytes to a nitrogen oxide activity. As a result, the increase of a synthase activity is connected with the increase of deformability, and the activity decrease with its decrease. The in-vitro research carried out by the authors (Korbut & Gryglewski, 1993) show that both too low and too high nitrogen oxide concentration heve caused erythrocytes rigidity. As it results from our earlier research, especially the erythrocytes which were exposed to an oxidation stress are, to a great extent susceptible to the rigidity through a peroxidation (Chmiel, Turczyński, Grabowska-Bochenek, Olszowy & Kuśmierski, 1999).

CONCLUSIONS

The stress evoked by water immersion lasting for about 4 hours evokes a slight decrease of erythrocytes deformability in a rat.

A blockage of a nitrogen oxide synthase during water immersion results in a comparatively considerable decrease of erythrocytes deformability in rats.

The increase of a nitrogen oxide activity during the stress evoked by water immersion results in a moderate erythrocytes deformability increase in rats.

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