found between MDA, CD, and Schiff bases, but an equal degree of correlation was found between the blood glucose level and concentrations of MDA and Schiff bases ($r = -0.64$, $p < 0.05$). In SHR with an extreme degree of adaptation to a permanently raised blood pressure (hereditarily determined) development of diabetes leads to failure of the adaptive reactions [2]; this may perhaps account for the great sensitivity of SHR to streptozotocin [8] and to the more severe course of diabetes as a whole.

The development of diabetes in normotensive and spontaneously hypertensive rats is thus accompanied by opposite changes in the intensity of LPO, possible evidence of failure of adaptation of spontaneously hypertensive animals during the development of diabetes.

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EFFECT OF ARGININE ON PROPERTIES OF ERYTHROCYTE MEMBRANES IN HYPOXIA

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UDC 615.31:547.495.9].015.4:[616.155.1-018.1:576 314]-092:612.273 2].076.9

KEY WORDS: hypoxia; arginine; erythrocyte membranes; lipid peroxidation; superoxide dismutase.

Potentiation of free-radical processes with an increase in the intensity of lipid peroxidation (LPO) is observed in hypoxia [2], and this, in turn, may cause many structural and metabolic changes, including changes in membrane permeability [12] and changes in activity of enzyme systems located in membranes [1]. Arginine has been shown to have a protective action in many functional states [9]. We have demonstrated the antihypoxic effect of arginine [12]. The influence of arginine on lipid metabolism has been demonstrated [3]. Its antiradical and antioxidant effects have been established by experiments in vitro [10].

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(Presented by Academician of the Academy of Medical Sciences T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 5, pp. 497-498, May, 1992. Original article submitted October 18, 1991.

0007-4888/92/0005-0668$12.50

The effect of hypoxia combined with administration of arginine on the physicochemical properties of erythrocyte membranes, on the intensity of LPO, and on activity of enzymes of antioxidant protection – superoxide dismutase (SOD) and catalase – was studied in the investigation described below.

EXPERIMENTAL METHOD

Female albino rats weighing 180-200 g served as the test object. Hypoxie hypoxia was induced in a continuous-flow hypoxic chamber by elevation to an altitude of 9000 m. Altogether four groups of animals were studied: 1) intact animals (control); 2) animals exposed to hypoxia for 3 h; 3) animals receiving about 1 ml of a solution of L-arginine hydrochloride, made up in physiological saline in a dose of 120 mg/100 g body weight, by intraperitoneal injection 0.5 h before the experiment; 4) animals receiving the above dose of arginine 3.5 h before sacrifice. The animals were killed by decapitation. Plasma was obtained by centrifugation of heparinized blood at 3000 rpm for 15 min. The erythrocytes were washed 3 times in physiological saline buffered with 10 mM Tris-HCl, pH 7.4. SOD [14] and catalase [8] activity was determined in a 10% hemolysate. Lipids were extracted from erythrocyte membranes, obtained by the method in [7], with a methanol:chloroform mixture [5]. The content of conjugated dienes (CD), primary LPO products [2], and Schiff bases, the end-products of LPO [3], was determined in the chloroform extract. The content of total lipids was determined by the sulfovanillin method [6] and the hemoglobin concentration in the hemolysate and plasma by the unified cyanhydrin method; protein was determined by Lowry's method. The structural state of the erythrocyte membranes was assessed by determination of the relative microviscosity of the lipid phase of the membranes by means of a fluorescent pyrene probe ("Sigma," USA), by the intensity of fluorescence of its excimers λ_{max} = 470 nm and monomers λ_{max} = 395 nm. The coefficient of excimerization was calculated by the equation $K = F_e/F_m$ [4]. The concentration of phospholipids in all samples was 0.1 mg/ml of erythrocyte suspension, and of pyrene 10 μmoles/ml. The phospholipid content was determined as phosphorus [6]. The significance of differences was estimated by Student's t test.

EXPERIMENTAL RESULTS

As Table 1 shows, the coefficient of excimerization fell significantly in hypoxia by 14.5%, indicating an increase in relative microviscosity of the zones of lipid–lipid contact in the erythrocyte membranes compared with the control. One possible cause of the increase in membrane rigidity could be intensification of LPO in the membranes. In fact, we found that after 3 h of hypoxia the concentrations of CD and Schiff bases (SB) was significantly increased by 31.7 and 36.4% respectively compared with the control. Consequently, in hypoxia LPO is intensified. This may perhaps account for changes in the physicochemical properties of the membranes. The erythrocyte membrane becomes more rigid and brittle in hypoxia, but in addition its permeability is increased, as is shown by an increase of 44.6% in the level of extraerythrocytic hemoglobin (EEH) in the blood plasma of the rats after 3 h of hypoxia. The plasma EEH level of the control animals was 12.6 ± 1.19 mg%. However, activity of enzymes of antioxidant protection (SOD and catalase) was virtually unchanged compared with the control, in both erythrocytes and blood plasma (Table 1).

Hypoxia thus has a damaging action on erythrocyte membranes. Injection of arginine before exposure to hypoxia prevents the increase in microviscosity of the erythrocyte membranes. The coefficient of excimerization of these animals did not differ significantly from the normal value. The CD and SB level also returned to normal. SOD and catalase activity in the erythrocytes was increased by 64 and 46% respectively compared with the control (Table 1). Plasma catalase activity of these animals also was increased by 53% compared with the control to 275.3 ± 18.3 μmoles H_2O_2/mg protein/min compared with 180.3 ± 15.7 μmoles H_2O_2/mg protein/min in the control ($p < 0.001$). Consequently, it can be tentatively suggested that one cause of normalization of the membrane LPO level may be activation of the enzymes of antioxidant protection. In animals receiving arginine before the session of hypoxia, stabilization of the erythrocyte membranes took place, as shown by the return of the plasma EEH concentration to the control level. The plasma EEH level in these animals was 12.8 ± 1.34 mg%.

Under hypoxic conditions arginine thus has an antioxidant action, stabilizing the structure and properties of the membranes and activating enzymes of antioxidant protection.

TABLE 1. Properties of Membranes, Level of LPO Products, and Activity of Enzymes of Antioxidant Protection in Erythrocytes of Rats Subjected to Hypoxia and Protective Action of Arginine

Parameter	Conditions			
	control	hypoxia	arginine + hypoxia	control + arginine
Coefficient of excimerization, K	0,55±0,014 (5) $p<0,001$	0,47±0,02* (9) $p<0,001$	0,58±0,01 (5) $p>0,1$	0,63±0,018* (5) $p<0,001$
Relative microviscosity, 1/K	1,82 (5)	2,13 (9)	1,72 (5)	1,59 (5)
Conjugated dienes, mmoles/mg lipid	5,59±0,34 (6)	7,36±0,45* (6) $p<0,001$	5,38±0,37 (6) $p>0,1$	4,51±0,33* (6) $p<0,02$
Schiff bases, units/mg lipid	6,62±0,35 (6)	9,03±0,33* (6) $p<0,001$	6,59±0,33 (6) $p>0,1$	5,72±0,29* (6) $p<0,05$
SOD, units/mg	15,5±2,3 (15)	11,1±1,41 (16) $p>0,1$	25,4±3,8* (12) $p<0,05$	28,8±3,54* (12) $p<0,01$
Catalase, nmoles H_2O_2/mg Hb/min	50,4±7,36 (12)	35,7±1,93 (12) $0,05<p<0,1$	73,6±9,92 (12) $0,05<p<0,1$	71,5±10,2 (12) $p>0,1$

Legend. Number of animals in series shown between parentheses. Asterisk indicates significant changes compared with control.

These properties of arginine also were exhibited when it was injected into the control animals. Their coefficient of excimerization was increased by 14.5% compared with intact animals, i.e., the relative microviscosity of the membranes was lowered and, consequently, they became more elastic. There was a parallel fall in the intensity of LPO: levels of CD and SB fell by 19.4 and 15.6% respectively, SOD and catalase activity were increased were increased by 86 and 42% respectively in the erythrocytes compared with the control. Plasma catalase activity was 264.1 ± 26.3 μmoles H_2O_2/mg protein/min, or 46% higher than the control level, However, membrane permeability was unchanged: EEH remained at the control level, namely 10.1 ± 0.95 mg% ($p > 0.1$).

Thus the antioxidant action of arginine, as revealed by experiments in vitro, is confirmed by experiments on animals also.

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