Specific Response of the Organism and Blood Leukocytes in Rats of Different Genetic Strains to Hypoxia

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By the open-field behavior, August rats were more resistant to acute hypoxia than Wistar rats. Hypoxic activation of the immune system was more pronounced in August rats. As differentiated from Wistar rats, the stress-limiting NO system in August rats was not suppressed during hypoxia. The effectiveness and resistance of this system to hypoxia were higher in August rats. Behavioral changes in Wistar rats under hypoxic conditions were accompanied by activation of HSP32 synthesis in blood leukocytes. This protein serves as an indicator of oxidative stress (*i.e.* adverse factor in hypoxia). August rats were more resistant to behavioral disturbances in hypoxia than Wistar rats. HSP32 synthesis in leukocytes from August rats was not impaired under hypoxic conditions. Our results indicate that variations in HSP32 synthesis in peripheral blood leukocytes can be considered as a matter of for resistance to acute hypoxia.

Key Words: *hypoxia; August and Wistar rats; blood leukocytes; nitric oxide; HSP32 proteins*

The rats of various genetic strains have different resistance to stress. These features are associated with genetically determined differences in the effectiveness of protective stress-limiting systems [6]. Differences in organism's resistance to stress are manifested in various reactions of peripheral blood leukocytes to stress exposure (model of the cellular stress response) [8]. These data are consistent with the fact that hypoxia and various diseases are accompanied by specific changes in blood cells [2,3, 9]. The observed changes reflect the state of the organism and effectiveness of therapy. It can be suggested that organism's response and function of blood leukocytes under conditions of hypoxia (important environmental factor) depend on genetically determined characteristics. Organism's response and function of peripheral blood leukocytes in August and Wistar rats with different resistance to acute stress were studied during acute hypoxia.

MATERIALS AND METHODS

Experiments were performed on adult male August and Wistar rats weighing 210.0±6.1 and 351.0±7.3 g, respectively. Acute hypoxia was induced in an altitude chamber for 30 min (7000 m above sea level). The effect of hypoxia was evaluated from changes in behavioral characteristics of rats in the open-filed (OF) test, variations in the weight of stress marker organs (adrenal glands; thymus, one of the central immune organs; and spleen, one of the peripheral immune organs), activity of the stress-limiting NO system, and intensity of lipid peroxidation (LPO). Activity of the NO system was estimated from the concentration of stable NO metabolites (nitrates/nitrites) in blood plasma. Experiments were performed with the agent reducing nitrates into nitrites. Nitrite concentration was measured by the

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Griess method. The response of peripheral blood leukocytes to hypoxia was studied by changes in the production of heat shock proteins HSP32 (selective indicators of oxidative stress) [10] and HSP70 (antioxidant and cytoprotective agents of the protective stress-limiting system) [11,12]. The intensity of LPO was estimated from the concentration of substances reacting with thiobarbituric acid (TBA) in blood plasma. A complex of TBA and malonic dialdehyde (MDA, main product of the TBA reaction) was studied quantitatively by measuring optical density of MDA on a KFK-3 photometer. MDA content was expressed in μmol/liter plasma. Leukocytes were incubated in a thermostat with the nutrient medium at 37°C for 7 h to study the synthesis of HSP32 in these cells.

HSP32 synthesis was assayed by the Western blot analysis. The animals were decapitated. The blood was rapidly sampled on ice and centrifuged at 3000 rpm for 15 min to obtain the plasma and cells.

RESULTS

In the OF test, horizontal activity of Wistar rats was higher than that of August rats (total number of crossed squares, Table 1). However, vertical activity of Wistar rats was much lower than that of August rats. Moreover, Wistar rats exhibited a lower number of explored holes than August rats. These results are consistent with published data [7]. We conclude that August rats are characterized by lower emotional reactivity and greater resistance to novelty (OF exposure) than Wistar rats. Hence, exploratory activity of August rats does not decrease under novel conditions.

Hypoxia was followed by a decrease in horizontal activity of rats in both groups. It should be emphasized that these changes were more pronounced in Wistar rats than in August rats (Table 1). Exploratory activity of Wistar rats sharply decreased under these conditions. The number of rearing postures in Wistar rats decreased by 3 times compared to the control. The number of holes explored by Wistar rats increased, but was lower than that in August rats. By the OF behavior, August rats were more resistant to hypoxia than Wistar rats.

After hypoxia the relative weight of the thymus in Wistar and August rats decreased by 15 and 35%, respectively, compared to the control (Table 2). These changes were similar to variations in the relative weight of the thymus under stress conditions. During stress, the relative weight of the thymus decreases more significantly in rats with low emotional reactivity (as compared to animals with high emotional reactivity). After hypoxia the relative weight of the spleen increased in Wistar rats (by 16%), but decreased in August rats (by 17%). Hence, hypoxia has various effects on the immune system in Wistar and August rats.

Involution the thymus in rats of both groups after acute hypoxia (30 min) is not related to the decrease in thymocyte number. Our conclusion is derived from the results of previous studies to estimate the total number of thymocytes after severe stress for 6-12 h [1]. It was shown that the number of thymocytes decreases only 3 h after 6-h stress and reaches minimum on day 1-2. Studying the state of lymphopoiesis and stages of lymphocyte differentiation in the immunogenetic system [4] suggests that the posthypoxic decrease in the weight of the thymus is associated with high-intensity migration of lymphocytes from this organ of lymphopoiesis into peripheral immune organs (*e.g.*, into the spleen) and peripheral blood. The posthypoxic increase in the weight of the spleen in Wistar rats can be related to increased migration of lymphocytes from the thymus. These changes are not accompanied by rapid release of lymphocytes into the blood. The weight of the spleen decreases in August rats, which indicates that migration of lymphocytes from central immune organs is accompanied by cell differentiation and release into the blood. Our results suggest that high resistance to

TABLE 1. Effect of Hypoxia on the OF Behavior (Locomotor Activity) of August and Wistar Rats (*M*±*m*)

Parameter	Wistar		August	
	control (*n*=10)	hypoxia (*n*=10)	control (*n*=8)	hypoxia (*n*=8)
Total number of crossed squares (horizontal activity)	62.60±4.00	24.70±2.97^{+}	44.71±6.43*	22.42±5.45^{+}
Total number of vertical rearing postures	12.10±1.51	3.80±0.91^{+}	16.27±1.10*	11.40±1.30*$^{+}$
Number of rearings with support	9.40±0.92	2.50±0.61^{+}	13.42±1.95	9.52±2.23*
Number of holes explored in OF	0.70±0.20	2.10±0.82^{+}	2.57±0.69*	2.67±0.97*

Note. Here and in Tables 2 and 3: *n*, number of animals per group. $p<0.05$: *compared to Wistar rats; $^{+}$compared to the control.

TABLE 2. Effect of Hypoxia on the Weight of the Thymus, Spleen, and Adrenal Glands in August and Wistar Rats (*M*±*m*)

Parameter	Wistar		August	
	control	hypoxia	control	hypoxia
Body weight, g	337.7±8.3 (*n*=9)	366.5±6.0 (*n*=10)	203.3±8.3* (*n*=7)	210.7±7.1* (*n*=7)
Weight of the thymus, g	0.550±0.035 (*n*=9)	0.482±0.029 (*n*=10)	0.235±0.017* (*n*=7)	0.163±0.012*+ (*n*=7)
Relative weight of the thymus, g/100 g body weight	0.164±0.014 (*n*=9)	0.131±0.007+ (*n*=10)	0.117±0.011* (n=7)	0.077±0.003*+ (*n*=7)
Weight of the spleen, g	1.275±0.045 (*n*=8)	1.61±0.05+ (*n*=10)	0.830±0.026* (*n*=7)	0.722±0.028* (*n*=7)
Relative weight of the spleen, g/100 g body weight	0.378±0.002 (*n*=8)	0.439±0.015+ (*n*=10)	0.413±0.025* (*n*=7)	0.343±0.015*+ (*n*=7)
Weight of the left adrenal gland, mg	23.87±1.37 (*n*=9)	25.28±0.52 (*n*=10)	13.96±0.50* (*n*=6)	16.04±0.90* (*n*=8)
Relative weight of the left adrenal gland, g/100 g body weight	7.026±0.297 (*n*=9)	6.932±0.191 (*n*=10)	6.895±0.232 (*n*=6)	7.608±0.258 (*n*=8)

hypoxia (according to the OF behavior) is associated with greater protective activation of the immune system in August rats than in Wistar rats. The weight of the left adrenal gland in August rats tended to increase after hypoxia (insignificantly). The weight of the adrenal glands in Wistar rats remained practically unchanged under hypoxic conditions (Table 2). Hypoxia was followed by activation of LPO in rats of both groups. After hypoxia MDA content in blood plasma from Wistar and August rats was 1.140±0.026 and 1.280±0.068 μmol/liter, respectively (*vs.* 0.930±0.097 and 0.940± 0.089 μmol/liter in control animals, respectively). No differences in MDA content were found in Wistar and August rats.

Hypoxia was followed by a sharp decrease in activity of the stress-limiting NO system in Wistar rats. These changes were manifested in a 3-fold decrease in the concentration of NO metabolites (nitrates/nitrites) in blood plasma (39.27±5.80 and 11.98±2.34 μmol/liter in control and treated animals, respectively). Hypoxia had little effect on the concentration of nitrates/nitrites in blood plasma of August rats (28.55±3.46 and 22.70±3.12 μmol/liter in control and treated animals, respectively). After hypoxia the concentration of NO metabolites in August rats was higher than in Wistar rats. These data indicate that posthypoxic behavioral disorders were accompanied by suppression of the protective stress-limiting NO system in Wistar rats. The greater resistance of August rats to hypoxia was associated with normal activity of the NO system.

TABLE 3. Effect of Hypoxia on HSP32 Synthesis in Blood Leukocytes from August and Wistar Rats (*M*±*m*)

Group		HSP32 concentration	
		ng/μg proteins	densitometric units
Wistar	control (*n*=4)	0.0285±0.0005	1.75±0.04
	hypoxia (*n*=5)	0.0340±0.0020+	2.11±0.17+
August	control (*n*=4)	0.0312±0.0006*	1.92±0.09*
	hypoxia (*n*=5)	0.0363±0.0040	2.09±0.20

Under control conditions, the concentration of HSP32 in August rats was much higher than in Wistar rats (Table 3). After hypoxia the concentration of HSP32 in leukocytes remained unchanged in August rats, but increased in Wistar rats (by 19% compared to the control, $p<0.05$). Hence, after hypoxia the concentration of HSP32 in leukocytes of Wistar rats was similar to that in August rats.

The study of the OF behavior showed that August rats are more resistant to acute hypoxia than Wistar rats. These differences were associated with greater activation of the immune system in August rats than in Wistar rats. Moreover, activity of the stress-limiting NO system in August rats was not suppressed during hypoxia. Hence, the effectiveness and resistance of this system to hypoxia were higher in August rats. Behavioral changes in Wistar rats under hypo-

xic conditions were accompanied by activation of HSP32 synthesis in blood leukocytes. This protein serves as an indicator of oxidative stress (*i.e.*, adverse factor in hypoxia). August rats were more resistant to behavioral disturbances in hypoxia than Wistar rats. HSP32 synthesis in leukocytes from August rats was not impaired under hypoxic conditions. Our results indicate that variations in HSP32 synthesis in peripheral blood leukocytes can be considered as one of the criteria for resistance to acute hypoxia.

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