
GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Synthesis of Heat Shock Proteins (HSP70) in Blood Leukocytes as a Criterion of the Resistance to Stress Injury

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The resistance of August rats to ulceration of the gastric mucosa induced by acute emotional stress was higher than in Wistar rats. August rats exhibited not only more potent activation of the protective nitric oxide system and mobilization of the immune system, but also increased synthesis of cytoprotective heat shock proteins HSP70 in blood leukocytes under stress conditions. Our results indicate that HSP70 protein synthesis in blood leukocytes during stress reflects organism's resistance to stress and, probably, to other adverse factors.

Key Words: *August and Wistar rats; emotional stress; blood leukocytes; HSP70 proteins; nitric oxide*

Pathological processes in the organism associated with hereditary predisposition to some diseases or observed under the influence of environmental factors involve peripheral blood cells. A large body of evidence exist that changes in blood cells reflects the state of organs and systems [4,7]. A correlation was found between the degree of inflammation, severity of asthma, and impairment of eosinophil apoptosis in asthmatics [5]. Previous observations showed that preoperative stress in humans is accompanied by a decrease in peripheral blood B lymphocyte count, which can be prevented by anti-stress therapy [12]. Hence, the stress response can be evaluated from the decrease in leukocyte count. The concentration of malonic dialdehyde and catalase activity in platelets reflect the level of physical exercise [3]. Inherited resistance of human organism to high-altitude hypoxia can be evaluated by

hypoxia-induced variations in the density of β -adrenoceptors on blood lymphocytes and the degree of their activation with isoproterenol [1,11].

We hypothesized that stress-induced changes in blood cells reflect the severity of injury and resistance or susceptibility of the organism to stress. Since activity of the stress-limiting system of cytoprotective heat shock proteins HSP70 reflects organism's response and severity of injury under various conditions [6,8,9], we compared HSP70 production in blood leukocytes under normal conditions and during acute emotional stress. The study was performed on August and Wistar rats differing in the resistance to stress. We also evaluated activity of the stress-limiting nitric oxide (NO) system in blood plasma.

MATERIALS AND METHODS

Experiments were performed on adult male August and Wistar rats of the same age. To produce stress the rats were placed for 30 min in a standard cage

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with water (22°C) and covered with a grid at a distance of 5 cm from the water surface [2]. The adverse effect of this procedure was evaluated by the severity of ulceration in the gastric mucosa, which serves as a criterion of stress injury.

The animals were decapitated 2 h after stress exposure. The blood was rapidly collected on ice and centrifuged for separation of the plasma and cell pellet. Ulceration of the gastric mucosa was evaluated by the number and area of ulcerative lesions. The thymus was weighted. Leukocytes were incubated in RPMI-1640 medium. Incubation was performed in a thermostat at 37°C for 7 h. Some leukocytes were exposed to heat shock in a thermostat at 42°C for 1 h and incubated at 37°C. Basal and heat shock-induced synthesis of HSP70 in leukocytes was studied by Western blot analysis. Activity of the NO system was estimated by the concentration of stable NO metabolites (nitrates and nitrites) in blood plasma. Nitrates were reduced to nitrites with a special reducer. Nitrite concentration was measured by the method of Griess.

RESULTS

Stress was accompanied by ulceration of the gastric mucosa (Table 1). The number of ulcers (per 1 stomach) in August rats was 2.3-fold lower than in Wistar rats. These data are consistent with the results of our previous studies that August rats are more resistant to acute stress than Wistar rats [10]. After acute stress, the relative weight of the thymus

decreased in August rats (by 16%), but remained unchanged in Wistar rats. The weight of the thymus and the total number of thymocytes were estimated previously under conditions of 6-12-h stress [4]. The decrease in the weight of the thymus after short-term stress (30 min) cannot be explained by a decrease in thymocyte count, because previous studies showed that the number of thymocytes decreases only 3 h after 6-h stress exposure and became most significant on days 1-2. The observed changes were probably associated with migration of lymphocytes from the thymus, which reflects compensatory increase in the number of peripheral blood cells as the immune response to stress [4]. The reaction of the thymus to acute stress in August rats was more pronounced than in Wistar rats. It can be hypothesized that the higher resistance of August rats to stress injury is accompanied by more pronounced defense activation of the immune system.

Basal HSP70 level in blood leukocytes from August and Wistar rats was not detected (Table 2, Fig. 1). The content of heat shock-induced HSP70 in leukocytes from control August rats was lower compared to Wistar rats. At the same time, stress sharply increased heat shock-induced HSP70 synthesis in leukocytes from August (by 87% of the control), but not from Wistar rats.

Thus we found that as compared to Wistar rats with low resistance to acute stress, stress-resistant August rats are characterized by more pronounced reaction of the protective stress-limiting system of heat shock proteins in blood leukocytes to stress

TABLE 1. Ulcerative Lesions of the Gastric Mucosa and Weight of the Thymus in August and Wistar Rats after Acute Emotional Stress ($M \pm m$, $n=10$)

Parameter		Rat strain	
		Wistar	August
Body weight, g	control	273±9	187±8
	stress	271±13	180±11
Weight of the thymus, g	control	0.653±0.064	0.249±0.008
	stress	0.582±0.032	0.201±0.014 ⁺
Relative weight of the thymus (thymus weight/body weight)	control	0.002368±0.000170	0.001335±0.000033
	stress	0.002156±0.000066	0.001123±0.000090 ⁺
Number of rats with ulcers	control	0	0
	stress	8	5
Number of ulcers per stomach	control	0	0
	stress	3.15±0.50	1.33±0.30 [*]
Area of ulcers per stomach, mm ²	control	0	0
	stress	2.36±0.60	0.22±0.09 [*]

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

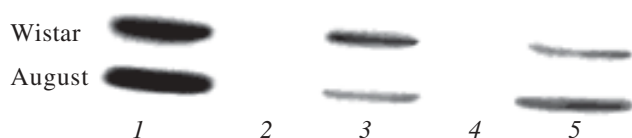


Fig. 1. Effect of stress on HSP70 synthesis in blood leukocytes of August and Wistar rats. HSP70 marker (1); control (basal synthesis, 2); control (heat shock-induced synthesis, 3); stress (basal synthesis, 4); stress (heat shock-induced synthesis, 5).

exposure (stimulation of HSP70 synthesis). Our results agree with published data that greater resistance of August rats to acute myocardial infarction is accompanied by more pronounced accumulation of HSP70 in the myocardium (compared to Wistar rats) [9].

Activity of the NO system and concentration of NO metabolites in blood plasma tended to increase in stressed Wistar rats. The 30% increase in the concentration of NO metabolites in these animals was statistically insignificant (Table 3). August rats exhibited significant activation of this system under stress conditions. The concentration of nitrates and nitrites in stressed August rats exceeded the basal level by 65%.

We showed that August rats more resistant to acute stress than Wistar rats exhibit more potent stress-induced activation of protective stress-limiting systems (HSP70 in leukocytes and NO system in blood plasma). The synthesis of protective stress proteins (HSP70) in blood leukocytes under stress conditions reflects organism, resistance to stress and, probably, to other adverse factors. Further studies should be conducted to develop new noninvasive methods for the diagnostics and correction of stress injury in humans.

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TABLE 2. HSP70 Synthesis in Blood Leukocytes of August and Wistar Rats after Acute Emotional Stress ($M \pm m$)

Series	HSP70 concentration in leukocytes	
	ng HSP70/ μ g proteins	densitometric units
Control, basal		
Wistar, $n=4$	0	0
August, $n=4$	0	0
Control, heat shock-induced		
Wistar, $n=6$	1.14 \pm 0.18	38.42 \pm 3.80
August, $n=6$	0.66 \pm 0.14*	18.35 \pm 2.60*
Basal, 30-min stress		
Wistar, $n=4$	0	0
August, $n=4$	0	0
Heat shock-induced, 30-min stress		
Wistar, $n=6$	0.96 \pm 0.18	33.57 \pm 5.60
August, $n=6$	1.24 \pm 0.30*	39.18 \pm 2.30*

TABLE 3. Concentration of Nitrates/Nitrites in Blood Plasma of August and Wistar Rats after Acute Emotional Stress ($M \pm m$)

Group	Wistar	August
Control	33.70 \pm 6.70 ($n=7$)	25.90 \pm 2.35 ($n=7$)
Treatment (stress)	43.90 \pm 2.30 ($n=7$)	42.90 \pm 7.61* ($n=6$)

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