GENERAL PATHOLOGY AND PATHOPHYSIOLGY

Synthesis of HSP70 in Blood Leukocytes as a Marker of Stress Resistance during Adaptation

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The effect of preadaptation to non-damaging emotional stress on the synthesis of HSP70 (stress-limiting factor) in peripheral blood leukocytes was studied in experiments on August and Wistar rats characterized by different sensitivity of the gastric mucosa to stress-induced injury. It was found that preadaptation improves stress resistance of Wistar rats characterized by lower innate resistance to acute mental stress and activates HSP70 synthesis in blood leukocytes. In August rats characterized by higher resistance to acute stress, adaptation reduced the resistance to stress-induced injuries, which was accompanied by the absence of activation of HSP70 synthesis in leukocytes compared to the level observed in nonadapted rats during acute stress. Thus, the intensity of HSP70 synthesis in peripheral blood leukocytes can serve as a marker of changes in animal resistance to acute stress caused by adaptation to non-damaging stress exposures and probably to other environmental factors.

Key Words: *blood leukocytes; adaptation to stress; emotional stress; HSP70; nitric oxide*

The role of genetically determined and acquired resistance to stress exposures in the pathogenesis, prophylaxis, and therapy of various diseases is now beyond doubts. In light of this, the development of methods for adequate evaluation of stress resistance in humans is an important problem. Pathological changes in humans and experimental animals involve peripheral blood cells and changes in these cells can serve as markers of changes in organs and systems [3,5,11,12]. During pathological states accompanied by immunodepression, blood lymphocytes are characterized by inability to activate the synthesis of cytoprotective proteins HSP70 in response to stress [10]. In rats, the intensity of HSP70 synthesis in peripheral blood leukocytes reflects innate resistance of the organism to acute emotional stress [8]. It is known that adaptation to environmental factors improves resistance to stress [4]. However, adaptation to acute stress improves stress resistance in animals with low genetically determined resistance to stress and decreases it in animals with high innate resistance. These changes are accompanied by changes in activity of stress-limiting systems of the organism, in particular stress proteins HSP70, during adaptation in animals with different genetically determined resistance to stress [6,9]. This suggests that different efficiency of the protective action of adaptation in animals with different innate resistance to acute stress can modify the state of peripheral blood leukocytes.

Here we evaluated HSP70 production in blood leukocytes during acute stress after adaptation to

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non-damaging stress exposures in August and Wistar rats characterized by different sensitivity to stress and plasma levels of stress-limiting factor NO modulating HSP780 synthesis during stress [10].

MATERIALS AND METHODS

Experiments were carried out on 2.5-month-old male Wistar and August rats. Acute emotional stress was modeled as follows: the rats were placed for 30 min in a cage with water (22°C) covered with a grid positioned 5 cm above the water [1]. The damaging effect of this exposure was evaluated by the size of ulcers on the gastric mucosa (GM) and thymus weight. Adaptation to stress was performed over 12 days (one session per day) according to the following scheme: session 1 for 1 min, session 2 for 3 min, session 3 for 5 min, sessions 4, 5, and 6 were similar to sessions 1, 2, and 3, respectively, sessions 7-12 lasted for 5 min each. Acute stress was modeled on the next day after completion of the adaptation session. The rats were decapitated 2 h after acute stress. The number and area of ulcerative lesions on GM were evaluated. The blood was rapidly collected after decapitation into ice-cold tubes and centrifuged for separation of plasma and cells. Leukocytes were incubated in RPMI-1640 in a thermostat at 37°C for 7 h: 1 h after the start of incubation, a portion of leukocyte suspension was subjected to heat shock (1 h in a thermostat at 42°C) and than incubation was continued at 37°C. Synthesis of HSP70 in leukocytes (basal and heat-shock induced) was measured by Western blotting. Activity of the NO system was evaluated by plasma concentration of stable NO metabolites nitrates/nitrites after reduction of nitrates to nitrites with a special reducer by the method of Griess. The data were processed statistically using Student *t* test.

RESULTS

Acute stress induced GM ulcers in non-adapted rats. In Wistar rats these lesions were more pronounced than in August rats (Table 1), which agrees with previous findings [6,7]. Preadaptation to stress considerably reduced the number and size of GM lesions after acute stress exposure in Wistar rats: the number and area of lesions in GM per one rat was lower than in non-adapted animals by 2 and 7 times, respectively. In August rats this adaptation had no protective effect and even slightly stimulated the formation of GM lesions during acute stress (Table 1). The adaptation itself induced ulcers of GM in August, but not in Wistar rats. In Wistar rats, acute stress exposure, adaptation, and stress against the background of adaptation induced no changes in the weight of the thymus (Table 1). In August rats, the weight of the thymus after acute stress decreased by 20% compared to the control, which confirmed our previous findings [8]. In August rats, preadaptation did not prevent changes in thymus weight during acute stress exposure: the weight of

TABLE 1. Effect of Preadaptation to Stress on GM Lesions and Thymus Weight during Acute Stress Exposure in August and Wistar Rats (*M*±*m*)

Group	Body weight, g	Number of rats with ulcers	Number of ulcers per rat	Area of ulcers per rat, mm^2	Thymus weight, g	Relative thymus weight (thymus weight/body weight)
Wistar						
control (*n*=20)	274±8	0	0	0	0.663±0.064	0.002368±0.00017
acute stress (*n*=20)	256.0±6.5	13	2.05±0.20	1.54±0.40	0.583±0.022	0.002156±0.00065
adaptation (*n*=20)	293.0±6.5	0	0	0	0.666±0.056	0.002253±0.000170
acute stress against the background of adaptation (*n*=10)	311.0±13.5	4	1.01±0.20^{++}	0.19±0.06^{++}	0.634±0.044	0.002018±0.000110
August						
control (*n*=15)	187.5±7.0	0	0	0	0.250±0.008*	0.001335±0.000030*
acute stress (*n*=18)	181.7±11.0	11	1.2±0.1*	0.27±0.05*	0.202±0.015^{+}	0.001124±0.000090^{+}
adaptation (*n*=9)	181±5	2	0.33±0.20	0.15±0.07*	0.218±0.011	0.0012055±0.0006100
acute stress against the background of adaptation (*n*=10)	186.0±5.5	6	1.1±0.3	0.32±0.07*	0.218±0.015	0.00118±0.00010^{+}

Note. Here and in Tables 2, 3: p<0.01-0.05 compared to: *Wistar rats, $^{+}$corresponding control, $^{++}$acute stress.

TABLE 2. Effect of Preadaptation to Stress on Concentration of HSP70 in Blood Leukocytes during Acute Stress Exposure in August and Wistar Rats (*M*±*m*)

Group		HSP70 concentration in leukocytes, ng/μg protein	
		basal	heat-shock-induced
Control	Wistar (*n*=6)	0	0.99±0.12
	August (*n*=5)	0	0.17±0.05*
Acute stress			
	Wistar (*n*=6)	0	0.91±0.10
	August (*n*=6)	0	0.42±0.08+*
Adaptation	Wistar (*n*=5)	0	0.92±0.11
	August (*n*=5)	0	0.18±0.07*
Stress against the background of adaptation			
	Wistar (*n*=6)	0	1.24±0.08++
	August (*n*=6)	0	0.41±0.09+*

the thymus in adapted and non-adapted August rats was below the control. Thus, preadaptation of August rats did not prevent the development of stress-induced GM ulcers and changes in thymus weight.

Induced content of HSP70 in peripheral blood leukocytes in control Wistar rats was higher than in control August rats (Table 2). Acute stress exposure induced no considerable changes in basal and induced content of HSP70 in leukocytes of Wistar rats, but considerably increased inducible content of HSP70 in August rats. Thus, increased resistance to acute stress exposure in August rats is associated with increased synthesis of cytoprotective proteins HSP70 in leukocytes, which confirms our previous findings [8]. In August and Wistar rats, adaptation to stress exposure did not change inducible and basal content of HSP70 compared to the control. In adapted Wistar rats, inducible content of HSP70 in leukocytes during stress increased compared to the level observed in non-adapted rats during stress. In adapted August rats, the level of inducible HSP70 in leukocytes after acute stress exposure did not differ from that in non-adapted rats during stress (Table 2). Thus, in adapted August rats, in contrast to Wistar rats, HSP70 synthesis in leukocytes is not activated during stress compared to the level observed in non-adapted August rats.

Thus, in Wistar rats the protective effect of preadaptation against gastric lesions during acute stress is associated with activation of HSP70 synthesis in leukocytes, which was not observed in non-adapted animals. In August rats, the absence of the protective effect of adaptation against gastric lesions is associated with the absence of elevation of HSP70 content in blood leukocytes in adapted rats during stress compared to that in non-adapted animals, *i.e.* activation of HSP70 synthesis during stress is absent. This can be explained by exhaustion of the HSP70 system in these animals during adaptation.

In Wistar rats, the stress exposure insignificantly increased the content of NO metabolites (nitrates/nitrites) in blood plasma, *i.e.* activated the NO system (the increase in metabolite concentration was statistically insignificant and we can speak about a tendency only). In August rats we observed significant activation of NO system during stress: the concentration of NO metabolites increased by 65% compared to the control. Thus, higher (compared to Wistar rats) hereditary resistance to acute stress in August rats is associated with activation of NO system during stress, which was not observed in Wistar rats. In experiments with preadaptation, no correlation between plasma content of nitrites/nitrates (*i.e.* activity of NO system) and the degree of the effect of preadaptation on the resistance to acute stress was revealed in both August and Wistar rats (Tables 1 and 3), which probably requires further investigation.

Thus, we demonstrated that the intensity of synthesis of HSP70, a stress-limiting factor, in peripheral blood leukocytes can serve as a marker of not only innate, *i.e.* genetically determined, but also modulated under the effect of adaptation to stress factors (and probably, other factors) resistance to acute emotional stress.

TABLE 3. Effect of Preadaptation to Stress on Concentration of NO Metabolites (Nitrates/Nitrates) in Blood Plasma during Acute Stress Exposure in August and Wistar Rats (μmol/liter, *M*±*m*)

Group	Wistar	August
Control	34.0±4.6 (*n*=6)	26.10±2.25* (*n*=7)
Acute stress	43.8±2.4 (*n*=6)	42.9±5.6+ (*n*=7)
Adaptation	50.0±3.1+ (*n*=6)	42.4±5.8+ (*n*=6)
Acute stress against the background of adaptation	38.10±2.25* (*n*=7)	44.4±6.4+ (*n*=7)

Note. The number of animals is shown in parentheses.

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