Differences Between August and Wistar Rats in Stress Reactions and in the Development of Adaptation to Stress

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Control and acutely stressed August rats have corticosterone levels 62% and 15% higher, respectively, than their Wistar counterparts, indicating that the activity of stress-mediating hypothalamic-pituitary-adrenal system in August rats is higher. On the other hand, the intensity of stress reactions and, consequently, the degree of activation of this system in August rats are 40-50% lower, as is the blood level of creatine phosphokinase. During adaptation to stress, August and Wistar rats show a similar decrease in the stress reaction and in its damaging effects. However, judging from the blood corticosterone/insulin ratio, adaptation to stress in August rats coincides with intensification of catabolic processes and a reduction in the efficiency of energy production.

Key Words: *Wistar rats; August rats; stress; corticosterone; insulin; adaptation*

Adaptation to short-term stresses is characterized by disappearance of the stress reaction and by protective cross effects, which is manifested in augmented resistance of the organism not only to stress but also to anoxia, physical activity, heat shock, and other factors [6-8]. It has been shown that animals of different genetic lines differ in regard to changes occurring in the cardiovascular system and blood and brain contents of catecholamines during stress, i.e., these changes are genetically determined [2,8, 11]. This suggests that the intensity of stress reaction, its damaging effects, and the development of adaptation to stress and other adverse factors also depend on genetic characteristics. In the present study we checked this suggestion in experiments with August and Wistar rats which react differently to stress, as evidenced by changes in their cardiovascular systems [2,11].

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MATERIALS AND METHODS

The study was conducted on male August and Wistar rats (body weight 250±40 and 300±20 g, respectively) from the Stolbovaya Nursery of the Russian Academy of Medical Sciences. Rats of each genetic type were separated into four groups. Group 1 consisted of intact (control) rats, group 2 of rats exposed to acute stress once (stressed group), group 3 of stress-adapted rats (adapted group), and group 4 of rats exposed to acute stress once after adaptation (adapted and stressed group). Rats were adapted to stress by eight immobilization sessions (fixation in the supine position). An immobilization session lasted for 15, 30, 45, and 60 min per day on days 1, 2, 3, and 4, respectively; the subsequent four sessions were conducted on alternate days, each session lasting 60 min. Acute stress was produced by immobilizing rats once for 60 min, which results in the greatest changes in the cardiovascular system [2]. The adapted rats were exposed to such a stress one day after the adaptation had been completed.

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TABLE 1. Plasma Levels of Corticosterone (CS) and Insulin in Acutely Stressed and Stress-Adapted August and Wistar Rats

Group	Rats	Parameters			
		CS, μg/100 ml	Insulin, μU/ml	CS/insulin	SRI
I: Control	Wistar	24.20±1.57 (20)	15.99±1.68 (10)	1 (10)	-
	August	39.34±1.96 (17)*	15.60±1.35 (11)	1.45 (11)	-
II: Stressed	Wistar	60.05±3.32 (18)*	29.20±2.60 (7)*	1.01 (7)	2.48 (18)
	August	70.28±3.32 (19)**	22.01±2.03 (9)*	1.63 (9)	1.78 (17)
III: Adapted	Wistar	36.58±4.30 (10)*,**	19.60±1.60 (10)	1.06 (10)	-
	August	48.38±7.63 (8)**	15.25±2.22 (8)	1.59 (8)	-
IV: Adapted and stressed	Wistar	44.71±3.74 (9)**	17.32±2.30 (9)**	1.47 (9)	1.22 (9)
	August	50.20±9.38 (8)**	12.86±1.62 (8)**	1.91 (8)	1.03 (8)

Note. SRI = stress reaction intensity defined as the ratio of the CS level in acute stress to the baseline level. Here and in Table 2: figures in parentheses are the numbers of rats in each group; differences are significant in comparison with Wistar rats (*), with group *I* (*), and with group *II* (**).

In animals of all four groups, blood concentrations of corticosterone (CS) and insulin were measured fluorometrically [10] and in a radioimmunoassay using standard RIO-INS-PG-^{125}I kits (Minsk, Belarus). The ratio of the CS concentration after acute stress to its concentration before stress (basal level) was used as an index of the stress reaction intensity. The rate of catabolism and the efficiency of the energy-supplying systems in stressed animals were evaluated by the CS/insulin ratio whose increase reflects increased intensity of catabolic processes and reduced efficiency of energy production [9,15]. The CS/insulin ratio was calculated as the ratio between the concentrations of CS and insulin expressed in percentage of their levels in the control Wistar rats, which were taken as 100% [3,9]. The damaging effects of stress reaction were assessed from changes in the activity of blood creatine phosphokinase (CPK), which was determined using Labsystems Company kits. Blood samples (2 ml) were collected from tail vessels (after incising the tail tip) into test tubes containing 50 μl EDTA (9%) on ice and centrifuged with cooling for 15 min at 3500 rpm. Plasma was rapidly frozen and stored at -24°C until use.

RESULTS

The mean CS concentration in the control August rats (i.e., basal CS level) was 62% higher than in the Wistar controls (39.34±1.96 *vs.* 24.20±1.57 μg/100 ml; $p<0.01$) (Table 1, *I*); August rats also had a significantly higher CS concentration after acute stress (Table 1, *II*). On the other hand, the intensity of stress reaction in August rats was lower (1.78) than in Wistar rats (2.48).

During adaptation, the CS level declined in rats of both strains and did not differ significantly from that in control animals by the end of adaptation ($p>0.1$) (Table 1, *III*). Acute stress induced much weaker stress reaction in all adapted rats than in non-adapted ones, so that the CS concentration was not significantly changed after stress in adapted animals (Table 1, *III* and *IV*), which is consistent with previous observations [3,4,8]. It is important to note that the substantial difference in CS concentration between August and Wistar rats observed in control groups disappeared almost completely during adaptation.

Table 1 shows that August and Wistar rats of all four groups did not significantly differ in the insulin levels. Acute stress caused a significant rise of insulin in all unadapted animals (Table 1, *II*). Although adaptation by itself had little or no effect on the insulin level, acute stress raised insulin only in unadapted rats (Table 1, *IV*), which is a consequence of the less intensive stress reaction and is characteristic of adapted animals in general [3,8].

The above-mentioned changes in CS and insulin levels led to changes the CS/insulin ratio. As shown in Table 1, August rats of each group had a much higher CS/insulin ratio than Wistar rats, suggesting that the energy-supplying system in August rats is in a state of "tension" even under conditions of relative rest and that stress appears to increase the tension. This situation remained in adapted August rats, suggesting that adaptation was inadequate in them.

August and Wistar rats showed no significant differences in the basal levels of CPK (Table 2, *I*). In unadapted rats of both strains, acute stress led, as expected, to a sharply increased CPK release into the blood, but the CPK level in August rats was 37% lower ($p<0.001$) (Table 2, *II*). Under the influence of adaptation, the CPK level decreased only in Wistar rats, indicating their augmented resistance to stress-inducible injuries. In adapted rats of both strains, the increase in the CPK release in response to acute stress was almost 3 times lower than in un-

TABLE 2. Plasma Creatine Phosphokinase (CPK) Activity (mU/liter) of Acutely Stressed and Stress-Adapted August and Wistar Rats

Group	Wistar rats	August rats
I: Control	186.8±18.0 (10)	148.8±14.6 (10)
II: Stressed	902.6±85.7 (9)*	572.5±34.2 (9)**
III: Adapted	115.1±10.1 (9)*	163.6±19.2 (8)*
IV: Adapted and stressed	321.5±49.5 (9)**	183.6±52.7 (8)***

adapted animals. This difference reflects the protective effect of developed adaptation. The CPK level in August rats remained significantly lower than in Wistar rats (Table 2, *IV*).

This study has revealed the following major differences in August and Wistar rats. First, both the basal activity of the stress-mediating hypothalamic-pituitary-adrenal system and its activity in stress are higher in August rats. However, the degree to which this system is activated in stressed August rats and, hence, the intensity of their stress reaction are lower, as indicated by the higher blood levels of catecholamines [2] and CS both before and after stress, and by the finding that the increments in CS and catecholamine levels in response to stress were lower than in Wistar rats. This may be a consequence of genetically determined differences between the two strains in the basal activities of the stress-mediating (hypothalamic-pituitary-adrenal) and stress-limiting (e.g., GABAergic, opioidergic, and prostaglandin) systems and in the ability of these systems to be activated in response to stress.

Second, the effects of the stress reaction in August rats are less damaging than in Wistar rats because of its lower intensity. This is manifested as a less pronounced rise in the blood level of CPK (which is a marker of cell damage) in August rats in response to stress. Moreover, August rats had significantly higher blood level of catecholamines than Wistar rats. It was shown that the corticoadrenal system is activated and the production of corticosteroids increases to compensate for the augmented catecholamine release in stressful situations. Corticosteroids exhibit antioxidant and membrane-stabilizing activities and are capable of protecting cells from injuries of the catecholamine nature such as those observed in stress, ischemia, and other conditions [1,12-14]. One of the possible mechanisms responsible for the lower CPK level and for the lesser severity of injuries observed in August rats may be a compensatory increase in the production of CS and other corticosteroids.

Third, stress reactions and their damaging effects in August and Wistar rats are limited to approximately equal degrees in the course of repeated stressful exposures, suggesting that adaptation to stress in these two strains is similar. However, as our analysis of changes in the CS/insulin ratio shows, the enhancement of catabolic processes and the reduction in the efficiency of energy-supplying systems in August rats during the development of adaptation to stress may be much more pronounced than in Wistar rats. This assumption agrees with the observations that August rats (compared with their Wistar counterparts) produce much smaller amounts of cell-protecting proteins (hsp70) during adaptation to stress and that the phenomenon of adaptive stabilization of cellular structures [5] due to activation of protein synthesis (a phenomenon that consistently develops during such an adaptation [6,7]) is less pronounced in August rats.

The results of this study emphasize the important contributions which genetically determined characteristics of the stress-mediating and stress-limiting systems make to the development of stress reactions and of adaptation to environmental factors.

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