Adaptation to Stress Improves Resistance to Gastric Damage during Acute Stress in Wistar Rats and Decreases Resistance in August Rats: Role of Serotonin

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 10, pp. 383-386, October, 2002
Original article submitted June 25, 2002

In August rats more resistant to acute stress-induced gastric damage than Wistar rats, preadaptation to nondamaging stress exposure did not prevent damage and even potentiated these damages. By contrast, in Wistar rats such adaptation decreased gastric damage caused by acute stress. Higher initial resistance of August rats to stress damage was associated with higher serotonin level and lower norepinephrine/serotonin ratio in the gastric mucosa than in Wistar rats. The negative effect of adaptation in August rats was associated with decreased serotonin level and increased norepinephrine/serotonin ratio in the stomach during stress. In Wistar rats exposed to stress the protective effect of adaptation was associated with an increase of serotonin content and a decrease of the norepinephrine/serotonin ratio in the stomach. Hence, the degree of resistance to stress-induced gastric damage can be due to genetically determined serotonin level and norepinephrine/serotonin ratio in the stomach.

Key Words: *August rats; Wistar rats; stress; adaptation; gastric ulcer; norepinephrine; serotonin*

August rats are more resistant to gastric damage induced by acute stress than Wistar rats [7,10]. Differences between these strains in the resistance to stress-induced injuries are largely determined by genetic peculiarities of the regulatory systems limiting the stress reaction and its damaging effects (nitrogen oxide, NO, and central dopaminergic system) [8-10]. Preadaptation to high-altitude hypoxia notably reduced these injuries in Wistar rats and little or not at all decreased them in August rats [7,8], which could be explained by different reaction to this mode of adaptation of the stress-limiting regulatory systems in August and Wistar rats [8,9]. It seems that rats of different genetic populations possess different capacity to adapt to environmental factors and this can be due to adaptation-induced changes in the regulatory systems responsible for stress reaction. In order to verify this hypothesis, we investigated the effect of adaptation to nondamaging emotional stress on the resistance to gastric injuries during acute damaging stress in August and Wistar rats and evaluated the status of the serotoninergic and noradrenergic systems in the gastric mucosa, as serotonin (5-HT) [5,6,11,13] and norepinephrine (NE) [1,12] are involved in the pathogenesis of stress-induced gastric ulcers.

MATERIALS AND METHODS

The study was carried out on male August (224±10 g) and Wistar rats (322±12 g). Stress exposure was carried out as described previously [2]. The rats were placed in a cage covered with wire net and the cage was put in water (21°C). Short-term adaptation consisted in 6 sessions: 1 min on day 1, 3 min on day 2, and 5 min on day 3; after 2-day interval the 3-day cycle was repeated. A course of prolonged adaptation consisted of 12 sessions (*i.e.* 4 above-described 3-day cycles). Four experimental series on August and Wi-

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0007-4888/02/1344-0329$27.00

star rats were carried out for each type of adaptation: control (intact rats); stress (acute damaging stress exposure); adaptation to stress; and acute damaging stress exposure after a course of adaptation.

Acute damaging stress was modeled by placing the rats after 24-h fasting into the cage for 30 min (short adaptation) or 1 h (long adaptation). Adapted rats were exposed to stress 24 h after the last adaptation session.

The rats were decapitated 2 h after acute stress, adapted controls (not exposed to stress) 24 h after the last adaptation session. The number and size of gastric lesions were evaluated and the content of NE and 5-HT in the gastric mucosa were measured. Fragments of the gastric mucosa were rapidly collected from the central zone of the greater curvature, and frozen in liquid nitrogen, stored until monoamine extraction with 0.1 M chloric acid (with 0.004% sodium hyposulfite) in 1:10 ratio (w/v). The tissue was homogenized in an Ultraturrax device, the homogenates were centrifuged at 15,000*g* and 4°C for 20-30 min. Supernatants were collected for analysis after filtration through 0.2-μ pore filters. The content of monoamines in the supernatant was evaluated by high performance liquid chromatography with electrochemical detection on an automated HP1050 complex and HP1049 electrochemical detector (Hewlett Packard); 125×4 mm Spherisorb ODS2 chromatographic column with 4×4 mm Lic hrospher 100RP-18 precolumn at the column temperature 35°C were used. The mobile phase was prepared on citrate phosphate buffer (20/70 mM, pH 3.0) containing 2 mM Na-EDTA; ion-pair reagent was 1.0 mM sodium heptyl sulfate and 0.5% trice distilled ethanol. Flow rate was 1.0 ml/min. The potential of glass carbon electrode was 550 mV compared to reference solid-state chlorosilver electrode. The reference monoamines were Sigma NE and 5-HT.

The significance of differences was evaluated using Student's *t* test.

RESULTS

A 30-min stress exposure induced less extensive gastric lesions in intact August rats that in Wistar rats (Table 1). These differences were more pronounced after 1-h stress: the number of gastric lesions per August rat was 3 times less than in Wistar rats and the area of lesions was almost 4-fold smaller (Table 1). These data are in line with previous reports [7].

Adaptation to stress, both short and long, did not induce gastric lesions in Wistar rats (Table 1), while in August rats it injured the gastric mucosa, though the lesions were less pronounced than after acute stress exposure. Moreover, preliminary adaptation to stress did not protect August rats from stress-induced injuries, but even potentiated them. The area of gastric ulcers per stomach after acute stress was 60% larger in August rats after short adaptation to stress than in intact animals exposed to similar stress (Table 1). In Wistar rats similar adaptation to stress prevented damage: it 2.3-fold decreased the number of lesions in the gastric mucosa after acute stress and 2.5-fold decreased their area in adapted animals compared to intact animals (Table 1). After prolonged adaptation

TABLE 1. Effect of Preliminary Short and Long Adaptation to Stress on Gastric Ulcers Induced by Acute Stress in August and Wistar Rats (*M*±*m*, *n*=8-10)

Parameter		Short adaptation (30 min)			Prolonged adaptation (1 h)		
		stress	adaptation to stress	adaptation+ stress	stress	adaptation to stress	adaptation+ stress
Number of rats with ulcers per group							
	August	8	3	7	4	3	8
	Wistar	9	0*	8	7	0*	6
Number of ulcers							
per group	August	26	5	14	15	4	18
	Wistar	29	0	13	57	0	16
per rat	August	2.6±0.6	0.53±0.20+	1.5±0.3	1.90±0.17	0.50±0.25+	2.25±0.37+
	Wistar	2.9±0.3	0*	1.3±0.2+	7.1±1.3*	0*	2.28±0.57+
Ulcer area per stomach, mm^2							
	August	0.70±0.12	0.04±0.04+	1.12±0.15+	0.88±0.12	0.05±0.02	1.28±0.14+
	Wistar	2.52±0.30*	0*	1.01±0.20+	7.3±0.6*	0*	1.28±0.60+

Note. All parameters are zero in control August and Wistar rats. The differences are significant in comparison with: *August rats, +stress group.

TABLE 2. Effect of Damaging Stress Exposure (30 min) on the Concentrations of 5-HT and NE (ng/mg Tissue) in the Gastric Mucosa of August and Wistar Rats Nonadapted and Adapted to Nondamaging Stress during 6 Sessions ($M \pm m$, n=9-10)

Parameter		Control	Stress	Adaptation+stress
5-HT	August	10.1±0.5	11.9±0.6*	7.90±0.61^{+}
	Wistar	6.01±0.70^{o}	6.4±0.1^{o}	7.3±0.4^{+}
NE	August	0.86±0.08	0.70±0.09	0.54±0.07*
	Wistar	0.98±0.11	0.88±0.16	0.50±0.06
NE/5-HT	August	0.090±0.007	0.060±0.002*	0.070±0.009
	Wistar	0.22±0.02^{o}	0.16±0.01*^{o}	0.080±0.009^{+}

Note. Differences are significant in comparison with: *control; $^{+}$stress group; ^{o}August rats.

to stress these differences were more pronounced (Table 1).

Hence, August rats are more resistant to single stress exposure, while their capacity to adapt to a repeated stress and form antistress defense is lower. In Wistar rats the initial stress resistance is lower, while the capacity to adapt to stress, *i.e.* to form antistress defense in the course of adaptation, is higher.

The strains differed by the concentrations of 5-HT and the ratio of NE/5-HT concentrations in the gastric mucosa. In August rats the initial level of 5-HT and the level during stress was 68 and 85% higher, respectively, than in Wistar rats (p<0.001, Table 2). However there were no significant differences in NE levels between August and Wistar rats, and therefore NE/5-HT ratios (initial and during stress) were essentially lower in August rats than in Wistar rats (Table 2). Hence, higher initial resistance of August rats to gastric injury in acute stress is associated with higher concentration of 5-HT and lower NE/5-HT ratio in the gastric mucosa than in Wistar rats. During acute stress gastric concentrations of 5-HT and NE decreased in adapted August rats in comparison with the levels in intact animals exposed to similar stress and did not differ from those in Wistar rats. Decreased resistance to acute stress-induced gastric injuries in August rats was associated with decreased 5-HT level in the gastric mucosa. By contrast, in Wistar rats exposed to acute stress the protective effect of adaptation to stress manifested in decreased number and area of stress-induced injuries (Table 1) was associated with increased 5-HT level in the gastric mucosa in comparison with the values in intact animals exposed to stress (Table 2). The protective effect of adaptation was associated with a 2-fold decrease of the NE/5-HT ratio in comparison with nonadapted rats (Table 2).

The role of 5-HT in the pathogenesis of stress-induced gastric injuries is not quite clear. On the one hand, central injection of 5-HT to mice (into the lateral cerebral ventricle) aggravated gastric lesions in stress, while intraperitoneal injection of 5-HT receptor blockers decreased these lesions. The stimulatory effect of 5-HT on gastric lesions differs in mice of different strains [5,6]. On the other hand, intraperitoneal injection of 5-HT and its precursor d,1-5-hydroxytryptophane reduced stress-induced ulcer formation [3], while increased 5-HT concentration in the gastric mucosa due to utilization of exogenous monoamine accelerated healing of stress-induced injuries [4]. It was shown that ulcer formation in stress was paralleled by a decrease in 5-HT level in the stomach [14]. It was found that injection of 5-HT receptor antagonists augmented stress-induced lesions in the stomach, while the protective effect of prostaglandin E_2 preventing these injuries was associated with an increase in 5-HT content in the stomach [11].

Our results are in line with the data on the protective role of endogenous 5-HT and suggest, together with these previous data, that genetically determined peculiarities of the serotoninergic system plays an important role in the mechanism of genetically determined differences in the resistance to stress and in the capacity to adapt to stress factors in August and Wistar rats.

The study was supported by the Russian Foundation for Basic Research (grant No. 99-04-48823).

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