PHYSIOLOGY

Stress Reactions in Rats during Immunization to Serotonin

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We studied the effect of immunization with a serotonin-bovine serum albumin conjugate on parameters of stress reaction to immobilization stress in rats. Active immunization was accompanied by changes in parameters reflecting animal resistance to emotional stress. The observed changes can be interpreted as a decrease in individual resistance to emotional stress. Active immunization of rats with a serotonin-bovine serum albumin conjugate was accompanied by production of autoantibodies against serotonin and dopamine. The role of autoantibodies against dopamine in modulation of the effect of immunization with serotonin-bovine serum albumin conjugate on the stress reaction in rats is discussed.

Key Words: *emotional stress; serotonin; behavior; rats; immunization*

Stress reactions in animals are mainly determined by specific integration of neurochemical mechanisms [6-8]. Peculiarities of serotoninergic processes in the hypothalamic paraventricular nucleus and dorsal hippocampus were demonstrated in rats with different resistance to stress [9]. Immunization of animals to neurotransmitters holds much promise for the correction of neurotransmitter systems in the brain. A positive effect of antiserotonin antibodies on the model of alcohol and opium syndromes was demonstrated [5].

Here we studied the stress reaction in rats under conditions of immunization to serotonin.

MATERIALS AND METHODS

Experiments were performed on 43 male Wistar rats weighing 250-300 g. The animals were kept under standard conditions and had free access to water and food. Emotional stress in rats was produced by immobilization in plastic cages and stochastic electrocutaneous stimulation for 1 h. The strength of stimulation was selected by the vocalization threshold. The rats were killed 1 h after stress. The adrenal glands, thymus, and spleen (marker organs) were taken. The gastric mucosa was examined for ulcers. The blood was sampled to measure the concentration of antibodies against serotonin and dopamine.

The rats were routinely immunized with a conjugated antigen of serotonin and bovine serum albumin (BSA) [1]. Serotonin-BSA conjugate (2 mg/kg) with complete Freund's adjuvant (1:1, total volume 0.5 ml) was injected subcutaneously into 2 sites on the back. Repeated subcutaneous injection of a conjugate (10 mg/ml) and incomplete Freund's adjuvant (1:1, total volume 0.5 ml) was performed 2 weeks after the first treatment. The study was conducted 1 week after the last injection. The intensity of production of antiserotonin antibodies was estimated by solid-phase enzyme immunoassay on a Dyna-

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tech mini-reader. A conjugate of serotonin and heterologous protein carrier (equine γ-globulin) was synthesized as described elsewhere and used as the test antigen [1]. The concentration of autoantibodies against serotonin was measured in rat plasma.

The open-field behavior was studied over 5 min before immunization and 23 days after the first test. During the second testing we evaluated the effect of immunization on locomotor, exploratory, and vegetative activity. The open field was a round area (diameter 1 m) illuminated with a 60 W lamp positioned at a height of 1.5 m above the center of the area.

The rats were divided into 4 groups: group 1, immunized stressed animals (n=12); group 2, immunized nonstressed animals (n=12); control group 3, injection of physiological saline (according to the immunization scheme) and stress (n=11); and control group 4, nonimmunized nonstressed animals (n=8).

RESULTS

Immunization with serotonin-BSA conjugate induced production of antibodies against serotonin in the blood (titer 1:512-1:2024) and synthesis of autoantibodies against dopamine (titer 1:512).

The time of grooming in rats of groups 1 and 2 during the second test increased to a lesser extent than in groups 3 and 4 animals (Table 1). The number of boluses in immunized animals did not decrease in the second test. These animals exhibited a more significant increase in the latency of the first movement and entrance into the center (test II).

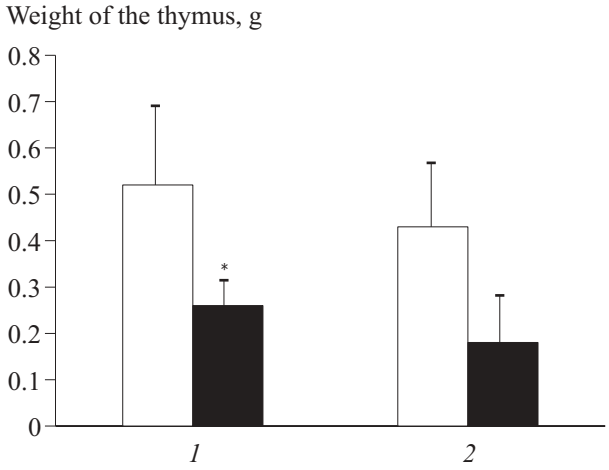

Fig. 1. Relative weight of the thymus in rats receiving physiological saline (light bars) and immunized with serotonin-BSA conjugate (dark bars). Nonstressed rats (*1*); 1 h after stress (*2*). *p<0.05 compared to animals receiving physiological saline.

In rats of groups 1 and 2 the relative weight of the thymus was lower (Fig. 1), while the relative weight of the adrenal glands was higher compared to animals of groups 3 and 4 (Fig. 2). The relative weight of the spleen did not differ in immunized and nonimmunized nonstressed rats (326.29±80.56 and 340.32±42.03, respectively). No differences in gastric ulceration were revealed in animals of groups 2 and 4 (Fig. 3). The relative weight of the thymus in rats of groups 1 and 3 was lower than in animals of groups 2 and 4 (statistically insignificant, p=0.08, Fig. 1). The relative weight of the adrenal glands in group 1 rats was much higher than in group 3 animals (Fig. 2). The relative weight of the spleen did not differ in rats of different groups. The num-

TABLE 1. Parameters of Rat Behavior in the Open-Field Test (n=12, $M\pm m$)

Parameter	Control		Immunization	
	before injection of physiological saline	after injection of physiological saline	before immunization	after immunization
Latency of the first movement, sec	5.17±4.32	8.583±5.530	7.08±5.43	11.83±4.65
Latency of entrance into the center, sec	61.00±40.23	90.20±38.68	78.33±53.54	170.00±48.65
Peripheral squares	46.083±21.450	28.50±22.89	45.92±21.13	32.33±25.69
Central squares	11.083±7.410	8.667±6.950	16.92±15.22	8.00±7.80
Peripheral rearing postures	16.5±10.5	8.75±7.10	15.83±10.20	7.83±6.98
Central rearing postures	1.917±1.680	0.4167±0.3400	2.33±1.88	0.4167±0.2300
Explored objects	12.00±5.86	5.333±4.440	16.33±10.85	7.00±5.65
Grooming, sec	28.25±15.46	68.33±37.33	20.92±12.16	32.67±22.34*
Fecal boluses	4.58±1.60*	3.58±1.24	4.92±1.90	5.00±1.50
Urination	0.8333±0.5200	0.9167±0.4000	1.25±0.82	1.083±0.700

Note. *p<0.05 compared to control rats receiving physiological saline.

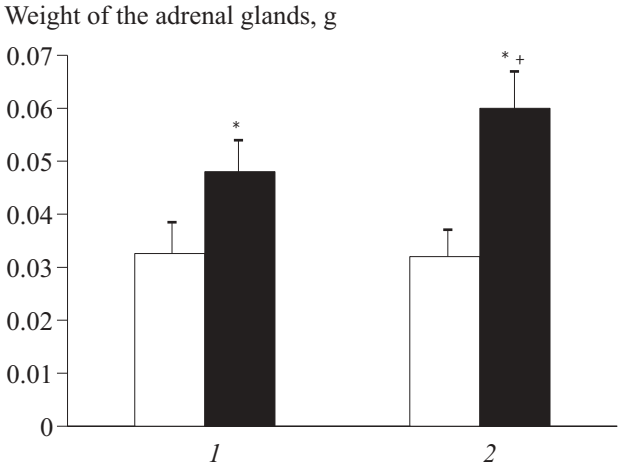

Fig. 2. Relative weight of the adrenal glands in rats receiving physiological saline (light bars) and immunized with serotonin-BSA conjugate (dark bars). Nonstressed rats (*1*); 1 h after stress (*2*). Here and in Fig. 3: $p<0.05$: *compared to rats receiving physiological saline; +compared to nonstressed rats of the corresponding group.

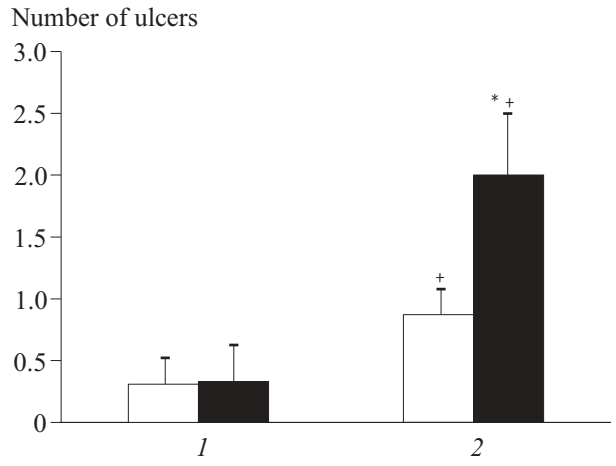

Fig. 3. Ulceration in the gastric mucosa of rats receiving physiological saline (light bars) and immunized with a serotonin-BSA conjugate (dark bars). Nonstressed rats (*1*); 1 h after stress (*2*).

ber of gastric ulcers in group 1 rats was much higher than in group 3 animals ($p<0.01$, Fig. 3).

Our results show that active immunization of rats with serotonin-BSA conjugate modified their adaptation to stress. The observed changes can be interpreted as a decrease in the resistance of animals to emotional stress. This conclusion is derived from the degree of stress-induced ulcers and more pronounced increase in the weight of the adrenal glands in immunized rats (compared to nonimmunized animals). It was probably associated with production of autoantibodies against dopamine. The mechanism of production of autoantibodies against dopamine probably includes reciprocal serotonin-dependent disturbances of dopaminergic processes in the central nervous system. Evaluation of the interaction between the serotoninergic and dopaminergic neurotransmitter systems showed that dopamine stimulated, while serotonin inhibited antibody production [4]. The influence of serotonin and catecholamines on behavioral characteristics of animals was described elsewhere [2].

Nonspecific stimulation of the immune system with BSA and Freund's adjuvant produced an opposite effect on the resistance of rats and mice to emotional stress (compared to immunization to serotonin) [3]. As distinct from immunization with serotonin-BSA conjugate, immunization of Wistar rats with BSA was followed by a significant increase in serotonin concentration in some brain structures and rise in dopamine content in the hippocampus [1]. The stress reaction in rats immunized with dopamine-BSA conjugate will be evaluated in further experiments.

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