GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Involvement of Autoantibodies to Neurotransmitters in Mechanisms of Stress Reaction in Rats

L. A. Vetrile, V. A. Evseev, A. E. Umriukhin*, A. N. Kravtzov*, S. V. Sotnikov*, and I. A. Zakharova

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Combined stress exposure of rats can lead to the formation of autoantibodies to neurotransmitters. Repeated stress is associated with the production of autoantibodies to serotonin, dopamine, norepinephrine, glutamate, and GABA. High emotional and behavioral reaction to stress is associated with intensive production of autoantibodies to serotonin, dopamine, norepinephrine, and glutamate.

Key Words: *autoantibodies to neurotransmitters; stress, neurotransmitters*

Mental stress develops under conditions of conflict situations, when the individual is unable to meet its basic requirements and achieve a useful adaptive result [7]. Primary changes in mental stress develop in the brain, and the complex of interactions between various immunoregulatory systems, including the neurotransmitter, neuropeptide, and glucocorticoid systems is essential for this process [1,4,9, 10,14]. The feedback mechanisms with their stress-limiting (protective) or dysregulatory (disordering the neuroimmune interactions) effects play an important role in these processes. Cytokines (IL-1β, IL-6, IL-18) [15] and autoantibodies to neurotransmitters are involved in the cerebral neurophysiological and neurochemical processes providing adaptation to stress. Enhanced production of autoantibodies to serotonin (5-HT), catecholamines, glutamate, and GABA was previously observed in experimental neuropathic painful syndrome and epilepsy (corasol kindling) [2].

Institute of General Pathology and Pathophysiology, the Russian Academy of Medical Sciences; *Institute of Normal Physiology, the Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** izaharova@mail.ru. I. A. Zakharova

Here we studied the possibility of production of autoantibodies to 5-HT, dopamine, norepinephrine, glutamate, and GABA and evaluated their role in manifestations of psychoemotional stress syndrome.

MATERIALS AND METHODS

Experiments were carried out on 36 male Wistar rats (250-300 g) kept under standard vivarium conditions at natural illumination with free access to water and food. After 1-week adaptation to conditions of a cage (10 animals per cage), basal behavioral activity of animals was tested in the open field test. The following parameters were evaluated: latency of the first movement and first visit to the center of the field, numbers of crossed squares, rearing episodes, and explored objects, numbers and duration of grooming acts, and number of fecal boluses. The summary activity score (SAS) was estimated, which comprised the number of crossed squares, rearing episodes, and explored objects. The rats were distributed into two groups by this score: with high (active) and low (passive) behavioral activities (Table 1). Stress exposure consisted

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in immobilization of rats on a platform with their limbs fixed for 1 h with simultaneous electrocutaneous stimulation, which was evaluated by vocalization threshold. Immobilization stress exposure was carried out twice with a 24-h interval. The rats were twice tested in the open field: 1 h and 14 days after stress exposure. After the experiment, the rats were decapitated and the blood was collected for detection of autoantibodies to 5-HT, dopamine, norepinephrine, glutamate, and GABA. Serum autoantibodies to neurotransmitters were measured by ELISA in polystyrene plates sensitized with the corresponding neurotransmitter—BSA test antigens, which were synthesized by the standard methods [10-12]. The content of autoantibodies was evaluated by optical density of the serum at λ=495 using a Mini-Reader (Dynatech) and expressed in arbitrary activity units as the K parameter: ratio of optical density of the serum from each experimental animal to the mean optical density of the serum from intact rat (n=10). K>1 indicated the presence of autoantibodies. The sera of experimental animals were also analyzed for autoantibodies to the carrier protein (BSA), which were not detected. Experiments were carried out in accordance with Regulations for studies on experimental animals, approved at P. K. Anokhin Institute of Normal Physiology and Institute of General Pathology and Pathophysiology; these regulations correspond to requirements of the World Society for Protection of Animals (WSPA) and European Convention for protection of experimental animals.

The results were statistically processed using Statistica software. The significance of differences was evaluated using Student's t test and Fisher's method.

RESULTS

Comparative analysis of behavioral activity of rats in the open field showed specific features of their reactions to stress (Table 1). SAS decreased in both groups 1 h after stress exposure. This decrease was more pronounced in passive rats; they were more sensitive to stress exposure than active animals. However, individual analysis showed different decrease in SAS after stress in active rats with initially high mean behavioral activity: in 4 of 10 animals this parameter decreased by 70%. This prompted dividion of all experimental animals into subgroups by the results of stress exposure (irrespective of their initial behavioral reaction). In subgroup 1, SAS decreased by 40% 1 h after stress, in subgroup 2 by 73% (Fig. 1). Two weeks after the stress exposure, behavioral activity of rats in subgroup 1 remained virtually the same, while in subgroup 2 SAS significantly increased (by 2.5 times; Fig. 1).

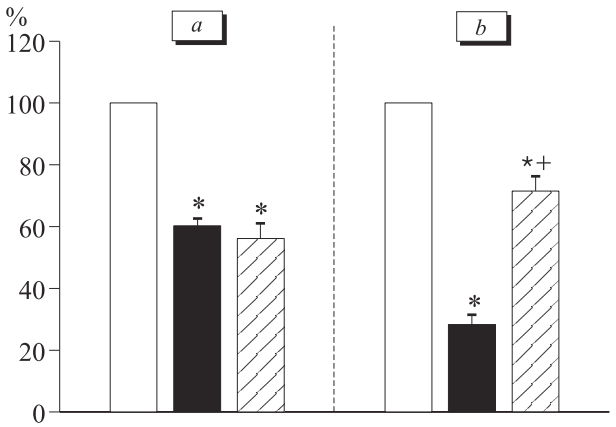

Fig. 1. Dynamics of motor activity of rats in subgroups 1 (*a*) and 2 (*b*) in the open field after immobilization stress. Ordinate: SAS. Light bars: initial SAS (before stress); dark bars: SAS 1 h after stress; cross-hatched bars: SAS 2 weeks after stress. **p*<0.001 compared to initial SAS; +*p*<0.002 compared to previous testing.

Comparative analysis of the intensity of antibody production to 5-HT, dopamine, norepinephrine, glutamate, and GABA 2 weeks after stress exposure showed significantly higher incidence of autoantibodies to these neurotransmitters (except GABA) in subgroup 2 rats (with more pronounced decrease in SAS after stress) than in subgroup 1 (Fig. 2). Rats less resistant to stress responded to it by more intense production of autoantibodies to 5-HT, dopamine, norepinephrine, and glutamate. This

TABLE 1. Motor Activity of Rats in the Open Field before and 1 h after Immobilization Stress

Parameter	Active rats		Passive rats	
	initial	1 h after stress	initial	1 h after stress
Number of crossed squares	105.0±2.19	51.5±7.4***	76.93±2.72++	21.1±3.7***
Number of rearing episodes	19.0±1.84	9.1±2.1**	13.33±1.35+	6.2±1.4**
Number of explored objects	2.58±0.82	3.0±1.5	2.93±0.78	0.3±0.2*
SAS	128.0±3.09	63.6±9.5***	93.2±3.09++	27.6±6.4***

Note. **p*<0.05, ***p*<0.01, ****p*<0.001 compared to initial values; +*p*<0.05, ++*p*<0.001 compared to active rats.

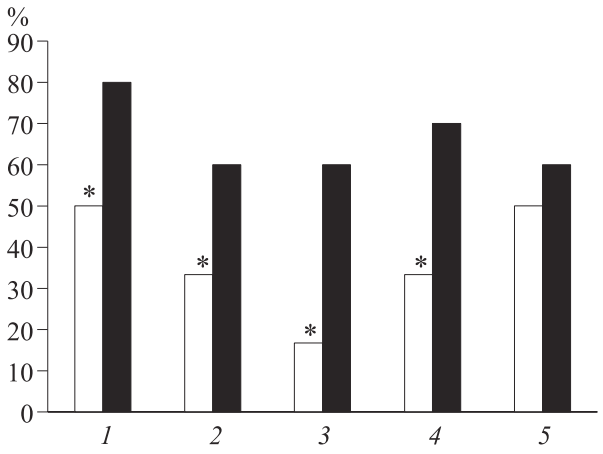

Fig. 2. Incidence of serum autoantibodies to neurotransmitters in rats with different degree of SAS reduction in the open field test. *1*) autoantibodies to 5-HT; *2*) autoantibodies to dopamine; *3*) autoantibodies to norepinephrine; *4*) autoantibodies to glutamate; *5*) autoantibodies to GABA. Light bars: 40% reduction of SAS in the open field (subgroup 1); dark bars: 73% reduction (subgroup 2). **p*<0.05 compared to the other subgroup.

result coincided with the results of analysis of the relationship between the incidence of autoantibodies to neurotransmitters and such emotional components of stress-induced reactions as the duration and number of grooming acts and number of fecal boluses. Antibodies to neurotransmitters were detected mainly in rats with high emotional reaction to stress (Fig. 3). It can be hypothesized that autoantibodies to 5-HT, dopamine, norepinephrine, and glutamate play a protective role. This can be seen from significant recovery of behavioral activity 2 weeks after stress exposure in subgroup 2 rats.

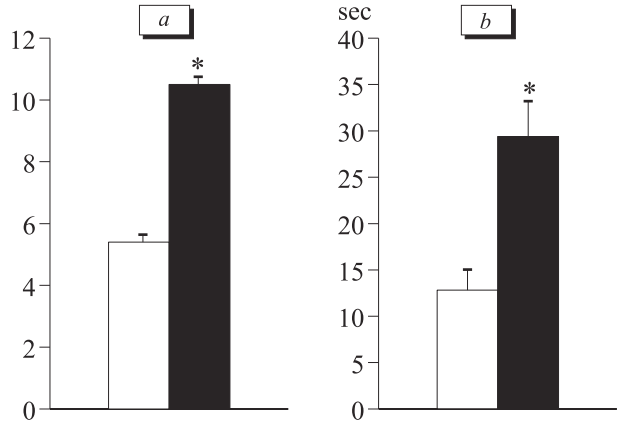

Fig. 3. Detection of autoantibodies to neurotransmitters and emotional component of total behavioral activity of rats in the open field. *a*) summary number of grooming acts and fecal boluses; *b*) total duration of grooming acts. Light bars: rats not reacting to stress by production of autoantibodies to neurotransmitters; dark bars: rats with autoantibodies to the studied neurotransmitters during the post-stress period. **p*<0.05 compared to the other subgroup.

Hence, evaluation of the results by the two main parameters (changes in behavioral activity and intensity of antibody production) showed that production of autoantibodies correlated with behavioral reaction (initial and post-stress activity). The highest production of autoantibodies to the majority of neurotransmitters was detected in rats whose behavioral activity parameters in the open field decreased significantly after stress and whose emotional reactivity was higher; in other words, these animals were more sensitive to stress, according to published data [3,6]. The increase in motor activity after 2 weeks in the group of stress-sensitive rats attested to the protective role of the detected autoantibodies to neurotransmitters.

The rats with high individual sensitivity to stress are characterized by high levels of serotonin in the cortex and brainstem [3], high dopamine content in the cortex, stem, and hypothalamus [3,5,6], and by norepinephrine deficiency in these brain structures [3,6]. Stress reactions are associated with changes in the levels and metabolism of neurotransmitters in the CNS; in turn, these neurotransmitters also act as the immune system regulators [1]. In our study, stress-sensitive rats responded to stress by more intensive autoantibody production. The protective effect of antibodies to dopamine on manifestations of the stress reaction was described previously [8].

Hence, the results indicate that psychoemotional stress syndrome leads to the development of an intricate complex of interactions between the nervous and immune system.

We detected the possibility of production of autoantibodies to neurotransmitters (serotonin, dopamine, norepinephrine, glutamate, and GABA) during combined immobilization stress in rats. The intensity of production of autoantibodies to neurotransmitters in stress depended on the behavioral reaction of animals: the highest production of autoantibodies to the majority of neurotransmitters was observed in animals most sensitive to stress. It seems that autoantibodies to neurotransmitters play a protective role in stress exposure, which is seen from the recovery of behavioral activity in stress-sensitive animals during the post-stress period.

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