Dynamics of Dopamine and Norepinephrine Contents in the Dorsal Hippocampus of Rats during Immunization with Dopamine Conjugate

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 4, pp. 374-377, April, 2007
Original article submitted September 13, 2006

The dynamics of dopamine and norepinephrine contents in the dorsal hippocampus of rats immunized with a dopamine-BSA conjugate was studied during immobilization stress by mean of the microdialysis technique. Immunization with dopamine conjugate was accompanied by intensive production of antibodies against dopamine in rat blood and a tendency toward an increase in dopamine content in the dorsal hippocampus even in the basal state (before stress exposure). Under stress conditions, dopamine content in the dorsal hippocampus of immunized rats significantly increased. In control rats, stress was accompanied by a significant increase in norepinephrine content in the dorsal hippocampus. The observed peculiarities in dopamine and norepinephrine contents in the dorsal hippocampus of rats immunized with a dopamine conjugate were typical of active animals more resistant to emotional stress.

Key Words: *stress; behavior; immunization; dopamine; norepinephrine; microdialysis*

Study of the central mechanisms of emotional stress and development of new methods for correction of individual resistance to stress are urgent problems of modern physiology [4]. Adaptation of the organism to stress is determined by a complex of neurophysiological mechanisms; an important role is played by processes taking place in the hippocampus [9]. Immunization of animals to neurotransmitters is a possible way for correction of neurotransmitter mechanisms [2].

We studied the dynamics of dopamine and norepinephrine contents in the dorsal hippocampus of rats immunized with a dopamine conjugate during emotional stress.

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MATERIALS AND METHODS

Experiments were performed on 50 male Wistar rats weighing 250-300 g. The animals were kept under standard conditions (daytime 8.00-20.00) and had free access to water and food. Stress was produced by immobilization (fixation of the limbs to a platform for 1 h) accompanied by moderate stochastic electrocutaneous stimulation. The strength of stimulation was selected by the vocalization threshold.

After 1-week adaptation, the rats were tested in an open field for evaluation of individual behavioral characteristics. According to open-field behavior, the animals were divided into 3 groups: rats with high, low, and intermediate exploratory activity.

0007-4888/07/1434-0399

A conjugated antigen of dopamine and BSA was synthesized as described elsewhere [1]. The rats were immunized according to a standard scheme. The 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. The second injection of the conjugate (10 mg/ml) with 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 production of antidopamine antibodies was measured by solid-phase enzyme immunoassay on a Dynatech mini-reader. Dopamine conjugated with heterologous protein carrier (equine γ-globulin) was synthesized as described elsewhere and used as the test antigen [1].

Microdialysis probes were implanted into the dorsal hippocampus of narcotized rats (400 mg/kg chloral hydrate) on day 23 after the start of immunization. Coordinates of the dorsal hippocampus were selected according to the atlas of rat brain [6]. We used concentric probes with a membrane pore size of 20 kDa. Dialysates were collected 48 h after surgery. Each dialysate was collected for 20 min. The probes were perfused for 2 h before the start of the study to achieve equilibrium between substances at both sides of the membrane. The scheme of the study included consecutive collection of 8 dialysate samples. The first 2 samples were collected under rest conditions. Then the rats were fixed to a platform (start of collection of the 3rd dialysate sample). The 3rd, 4th, and 5th samples were collected during stress and the 6th, 7th, and 8th samples were collected after the end of stress. The rats were decapitated 1 h after stress. The content of antibodies to dopamine in blood samples was measured.

The contents of dopamine, norepinephrine, and serotonin in dialysates were measured by HPLC with electrochemical detection using a LC-304T chromatograph (BAS, West Lafayette) equipped with a Rheodyne 7125 injector and 20-μl loop for sample application. Substances were separated on a Hypersil BDS C18 reversed-phase column (4 μ, 4.6×100 mm) and detected electrochemically using a LC-4B amperometric detector with a TL-5 well (BAS, West Lafayette). The study was performed at 0.85 V using an Ag/AgCl reference electrode.

The data were processed using Statistica 5.5 software. The significance of differences was estimated by analysis of variance and Student's *t* test.

RESULTS

The titer of antidopamine antibodies in the blood of rats immunized with dopamine-BSA conjugate varied from 1:512 to 1:4096. Dopamine content in the dorsal hippocampus of rats immunized with dopamine conjugate and control animals was measured under rest conditions (Table 1). Under rest conditions, the mean content of dopamine in 2 dialysate samples from the dorsal hippocampus of rats immunized with dopamine conjugate was higher than in control animals (3.26±1.27 *vs*. 1.76± 0.48 pmol/20 min, respectively). The content of norepinephrine underwent opposite changes. Under rest conditions, the mean content of norepinephrine in the first 2 dialysate samples from the dorsal hippocampus of immunized rats was slightly lower than in control animals (1.33±0.35 *vs*. 2.23±0.65 pmol/20 min, respectively, Table 1).

Immobilization stress was accompanied by different changes in the contents of dopamine and norepinephrine in the dorsal hippocampus of rats of both groups (Figs. 1 and 2). During the first 20 min of immobilization stress dopamine content in immunized rats significantly increased (8.092± 1.910 *vs*. 5.81±1.34 pmol/20 min under rest conditions, $p<0.05$). In nonimmunized rats dopamine content tended to decrease over the first 20 min of stress (5.64±1.78 *vs*. 5.82±1.84 pmol/20 min under rest conditions). Dopamine content in immunized rats was much higher than in control animals ($F_{(1.22)}=2.85$, $p<0.05$, Fig. 1). After the end of stress, dopamine content in rats immunized with dopamine conjugate remained at a higher level compared to that in control animals.

Immobilization stress was accompanied by changes in norepinephrine content in the dorsal hippocampus (Fig. 2). Over the first 40 min of stress,

TABLE 1. Neurotransmitter Content in the Dorsal Hippocampus of Control Rats and Animals Immunized with Dopamine Conjugate under Rest Conditions (pmol/20 min, *M*±*m*)

Parameter	Control group		Immunized rats	
	1st dialysate	2nd dialysate	1st dialysate	2nd dialysate
Dopamine	1.92±0.51	1.60±0.46	2.71±1.12	3.77±1.43
Norepinephrine	1.74±0.51	2.65±0.76	1.64±0.43	1.04±0.22

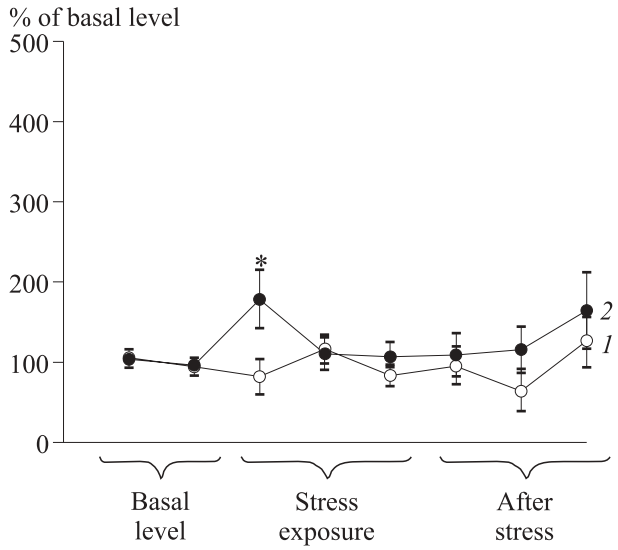

Fig. 1. Dopamine content in the dorsal hippocampus of control rats (*n*=10, *1*) and animals immunized with dopamine conjugate (*n*=18, *2*). Here and in Fig. 2: **p*<0.05 compared to the control group.

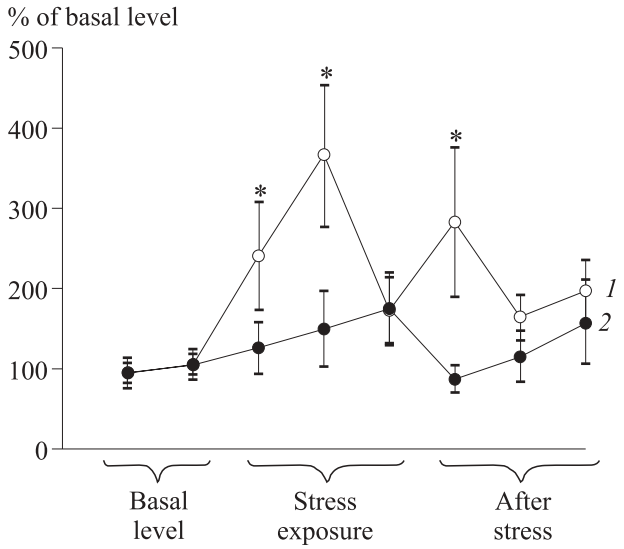

Fig. 2. Norepinephrine content in the dorsal hippocampus of control rats (*n*=9, *1*) and animals immunized with dopamine conjugate (*n*=18, *2*).

norepinephrine content in the dorsal hippocampus of control rats significantly increased and surpassed that in immunized animals ($F_{(1.17)}$=3.36, p<0.05, Fig. 2). Norepinephrine content tended to increase in immunized animals. Norepinephrine content in the dorsal hippocampus of control rats increased over the first 20 min after the end of stress (p<0.05 compared to the basal level, Fig. 2) and was much higher than in animals immunized with dopamine conjugate ($F_{(1.21)}$=8.36, p<0.01). Over 60 min after stress, norepinephrine content in the dorsal hippocampus of control rats remained higher than in immunized animals (Fig. 2).

Our results indicate that immunization with dopamine-BSA conjugate is accompanied by changes in the central dopaminergic and noradrenergic mechanisms of the immobilization stress reaction in the dorsal hippocampus. Under rest conditions, dopamine content in immunized rats with high blood titter of antidopamine antibodies is higher than in control animals. During stress exposure, dopamine content in the dorsal hippocampus significantly increases in immunized rats, but remains unchanged in nonimmunized animals. The stress-induced increase in norepinephrine content in the dorsal hippocampus was not observed after treatment with dopamine conjugate. During stress exposure and over the first 20 min after the end of stress, norepinephrine content in control rats was higher than in animals immunized with dopamine conjugate.

The effects of immunization with dopamine conjugate on central neurotransmitter mechanisms of the stress response in rats are probably related to the influence of antidopamine antibodies. The titer of these antibodies in the blood is high in immunized rats. The effect of peripheral antidopamine antibodies on dopamine content in the dorsal hippocampus, which is separated from the blood by the blood-brain barrier, can be associated with changes in the permeability of the blood-brain barrier under stress conditions [5]. Published data show that transitory hypertension induced by epinephrine injection (1 min) is followed by an increase in blood-brain barrier permeability for G antibodies, which can be detected in the brain parenchyma [10]. Moreover, polypeptides can migrate from the peripheral circulation into the brain tissue via circumventricular structures devoid of the blood-brain barrier [8].

Previous studies showed that thermal stimulation of the tail results in an increase in dopamine content in the medial prefrontal cortex of behaviorally active rats, which are characterized by low incidence of freezing behavior and short time of grooming [7]. High locomotor and exploratory activity is associated with individual resistance of animals to emotional stress [3]. The content of neurotransmitters in the dorsal hippocampus of rats immunized with dopamine conjugate and subjected to immobilization stress can illustrate central mechanisms underlying the increase in the individual prognostic resistance to emotional stress.

Our results on norepinephrine content in the dorsal hippocampus of rats are consistent with the notion that immunization with dopamine conjugate increases the individual resistance to emotional stress in rats. Evaluation of the role of norepineph-

rine in the stress reaction revealed a direct correlation between the stress response and increase in norepinephrine content in various brain structures. For example, rats characterized by severe ulceration in the gastric mucosa under conditions of unpredictable electrocutaneous stimulation exhibit a more significant increase in norepinephrine content in brain structures [12]. Previous studies demonstrate that significant activation of noradrenergic mechanisms in the brain under stress conditions is accompanied by severe ulceration and hyperactivation of the hypothalamic-pituitary-adrenal axis [11]. We showed that the reactivity of noradrenergic mechanisms in the dorsal hippocampus of rats immunized with dopamine conjugate decreases during immobilization stress. These changes probably underlie the increase in the individual resistance of animals to stress.

Immunization of rats with dopamine-BSA conjugate is accompanied by changes in the neurochemical mechanisms of the stress response in the dorsal hippocampus. Variations in the contents of dopamine and norepinephrine in the dorsal hippocampus of rats immunized with dopamine conjugate are typical of active animals more resistant to emotional stress. These central neurochemical mechanisms of the stress response in the dorsal hippocampus probably underlie the antistress effect of immunization with dopamine conjugate.

This work was supported by the Russian Foundation for Basic Research (grant No. 04-04-49047) and Foundation of the Presidium of the Russian Foundation for Support of Leading Scientific Schools in the Russian Federation (grant No. NSh-9825. 2006.7).

REFERENCES

1. L. A. Basharova, L. A. Vetrile, N. A. Bobkova, *et al*., *Patol. Fiziol. Eksp. Ter*., No. 1, 41-43 (1993).
2. V. A. Evseev, T. V. Davydova, V. G. Fomina, *et al*., *Byull. Eksp. Biol. Med.*, **116**, No. 12, 582-584 (1993).
3. E. V. Koplik, *Zh. Vestn. Nov. Med. Tekhnol*., No. 1, 16-18 (2002).
4. K. V. Sudakov, *Individual Resistance to Emotional Stress* [in Russian], Moscow (1998).
5. B. Esposito, N. Chandler, K. Kandere, *et al*., *J. Pharmacol. Exp. Ther*., **303**, 1061-1066 (2002).
6. O. Giorgi, D. Lecca, G. Piras, *et al*., *Eur. J. Neurosci*., **17**, No. 12, 2716-2726 (2003).
7. H. S. Haas and K. Schauenstein, *Prog. Neurobiol*., **51**, 195-222 (1997).
8. J. J. Kim, E. Y. Song, and T. A. Kosten, *Stress*, No. 9, 1-11 (2006).
9. F. Kuang, B. R. Wang, P. Zhang, *et al*., *Int. J. Neurosci*., **114**, 575-591 (2004).
10. D. A. Morilak, G. Barrera, D. J. Echevarria, *et al*., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **29**, 1214-1224 (2005).
11. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, New York (1998).
12. M. Tanaka, *Ind. Health*, **37**, 143-156 (1999).