Antistress Effect of Semax in the Course of Recovery of Spleen Lymphoid Structures after the Stress in Rats with Different Behavioral Activity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 615-617, May, 2012
Original article submitted March 2, 2011

We studied the effect of antistress peptide Semax, an ACTH4-10 analogue, on the cellular composition of spleen lymphoid structures in Wistar rats with different stress tolerance in the course of post-stress recovery (days 1, 3, 14, and 30). Preliminary administration of Semax alleviates stress-induced proliferation of macrophages and destructive processes in functionally active zones of the rat spleen on days 1, 3, and 14 after the stress exposure, which attests to its capacity to reduce the adverse effects of 1-h stress load on proliferation of macrophages and destructive processes in functionally active zones of this organ.

Key Words: *spleen; stress; immune system; Semax*

The spleen as an immune organ responds quickly and efficiently to changes in the environment, in particular to the impact of various stressors [7-9]. Semax, an ACTH4-10 fragment (ACTH4-7-Pro-Gly-Pro) analogue, is a regulatory peptide with prolonged action and produces an anti-stress effect [1-4]. Semax administered intraperitoneally for a long time increases the resistance to hypoxia and improves memory, attention, and learning capacities of experimental animals [2,5,10]. The preparation is used in clinical practice for the treatment of various CNS diseases (particularly acute disorders of cerebral circulation) and as an adaptogen for healthy people under extreme conditions [2].

Here we studied the effect of Semax as the antistress peptide during post-stress structural recovery of functionally active zones of the spleen in rats with different stress tolerance.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats (n=128) weighing 250-300 g.

Individual typological characteristics of rats were assessed in the open field test for 3 min [6]. Two groups were identified by the test results: active (n=64; activity index 2.5-7.5) and passive animals (n=64; activity index 0.2-0.8). Then, behaviorally active and passive rats were divided into the following 8 groups (~16 animals per group): intact control (group 1), administration of Semax (group 2); stress exposure (group 3), stress against the background of Semax (group 4).

Acute emotional stress was modeled by immobilization of the rats with simultaneous electrocutaneous stimulation [6,8]. The animals were placed into individual plastic boxes for 1 h; metal needle electrodes were fixed to the skin of the back. Electrocutaneous stimulation with alternating current was performed by a stochastic pattern with pulse length of 1 msec and voltage of 4-6 V at 50 Hz. Stimulation strength was adjusted individually according to the vocalization threshold in response to electrical stimulation. Each stimulation lasted for 30-60 sec; 16-18 stimuli were applied over 60 min.

Semax was administered intraperitoneally in a dose of 20 mg/kg immediately before placing the animals into the tight boxes and the start of stress exposure. The animals were sacrificed by decapitation 1 h and 3, 14, and 30 days after the stress exposure.

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Microscopic anatomy of functionally active zones of the spleen was studied on 5-6-μ transverse and longitudinal sections stained using standard histological techniques (hematoxylin and eosin, Heidenhain's hematoxylin, azan, by the method of Masson–Goldner). The significance of differences between the means was assessed by Student's *t* test.

RESULTS

Cell composition of the periarteriolar lymphoid sheaths (PALS) in the rat spleen in the control animals and passive rats was similar on days 1, 3, 14, and 30. The content of small lymphocytes in PALS in active and passive animals was 19.60±0.07 and 17.60±0.07 cells, respectively. Cells with signs of destruction were detected in a few cases on slides of the spleen in passive and active rats of control group. The content of macrophages in all rats in the control group was 0.60±0.04 and 0.90±0.04 cells, respectively.

Cell composition did not differ significantly from the control in active and passive rats after Semax administration without stress on day 1. The percentage of small lymphocytes in PALS of the spleen in active and passive rats on day 3 after intraperitoneal administration of Semax increased by 63.0±0.6 and 44.2±1.5%, respectively, in comparison with day 1. On day 1, cells with signs of destruction in the PALS of the spleen were found in rare cases in both passive and active animals. On day 3 after Semax administration, the content of these cells in active and passive rats increased by 1.4±0.4 and 4.5±0.5%, respectively, in comparison with day 1; the content of macrophages in PALS of active and passive rats increased by 2.6±1.3 and 2.5±0.5%, respectively. The same dynamics of cell composition was also recorded on experimental days 14 and 30 after peptide administration.

In the stress group, the percentage of small lymphocytes in the PALS of the spleen in active and passive animals 1 h after the stress exposure significantly decreased by 27.0±1.4 and 37.0±0.4% ($p<0.05$) in comparison with that in non-stressed animals. One hour after emotional stress, the percentage of cells with signs of destruction increased by 6.4±0.8% in passive rats and by 1.7±0.1% in active rats. The content of macrophages 1 h after the stress significantly increased by 4.8 times ($p<0.01$) in active and passive rats in comparison with that in non-stressed animals. On day 3 after the stress exposure, the percentage of small lymphocytes in PALS of the spleen in active and passive rats was significantly reduced by 1.4 and 1.2 times in comparison with day 1 ($p<0.05$). In passive rats, percentage of the cells exhibiting characteristics of destruction was increased by 8.4±2.8%; in active, by 3.7±1.5%. At the same time point, the percentage of the macrophages was significantly increased by 5.6 and 4.5 times in active and passive rats in comparison with the control group. On day 14 after the stress exposure, the amount of macrophages in active rats also significantly increased by 3.5 times in comparison with the control group. The percentage of small lymphocytes in PALS in all animals significantly decreased by 47.0±3.2 and 32.0±2.5% ($p<0.05$), respectively, in comparison with the controls. On day 30 after the stress exposure, the number of cells with signs of destruction in passive and active animals still slightly surpassed the control values.

In passive and active animals receiving Semax before emotional stress, the content of small lymphocytes on day 1 decreased by only 1.2 times in comparison with non-stressed animals treated with the peptide (Semax-control group). One hour after the stress exposure, the percentage of cells with signs of destruction in passive and active animals increased by 6.40±0.08 and l.70±0.06%, respectively, in comparison with the corresponding values in the Semax-control group. After Semax administration, the percentage of macrophages in passive and active rats was increased by 4.5±0.5 и 1.4±0.4%, respectively, in comparison with the Semax-control group.

In active rats receiving Semax before stress exposure, the percentage of small lymphocytes on day 3 was elevated by 43.6±1.5% and in passive rats this parameter practically did not differ from that in non-stressed rats receiving the peptide. The content of macrophages in passive and active rats on day 3 after stress preceded by Semax treatment did not differ significantly from that in the Semax-control group. The content of cells with signs of destruction in passive and active rats on day 3 after stress and Semax administration increased by 2.0 and 1.6 times, respectively, in comparison with the Semax-control group.

On day 14 after stress with prior administration of Semax, the percentage of small lymphocytes in active and passive rats returned to the control values (group 2). The stress against the background of Semax pretreatment increased the percentage of cells with destructive changes in passive and active animals by 3.0 and 2.5 times, respectively, in comparison with the Semax-control group. On day 14, the percentage of macrophages in PALS of active and passive rats exposed to stress against the background of Semax pretreatment returned to the values of the Semax-control group (group 2).

On day 30 after stress with prior administration of Semax, the percentage of small lymphocytes and cells with signs of destruction returned to the values of the Semax-control group in all rats. The amount of macrophages in the PALS of the spleen was increased 2.5 and 1.5-fold, respectively, in comparison with the Semax-control group.

Thus, preliminary administration of Semax to rats suppressed proliferation of macrophages and destructive processes in functionally active zones of the spleen (on days 1, 3, and 14 after the stress exposure). This suggests that the peptide can reduce the adverse effects of emotional stress. In active rats, this effect was slightly more pronounced than in passive ones.

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