Efficiency of Ultralow Doses of Antibodies to S100 Protein and Delta Sleep-Inducing Peptide in Rats with Anxious Depression

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We studied the effects of single peroral treatment with antibodies against S100 protein and delta sleep-inducing peptide in ultralow doses on behavioral characteristics of rats with anxious depression produced by acute stress (unavoidable electrical shock). Stress-produced behavioral changes and anxiolytic activity of antibodies were determined using the elevated plus-maze, open field, and tail suspension tests. High efficiency of the mixture of antibodies against S100 protein and delta sleep-inducing peptide was observed in all tests. Anxiolytic activity of anti-S100 antibodies (although less pronounced than that of the mixture of antibodies) was revealed in the elevated plus-maze and tail suspension test.

Key Words: *anxious depression; ultralow doses; antibodies to S100 protein; antibodies to delta sleep-inducing peptide; anxiolytic activity*

The preparation Proproten containing antibodies to S100 protein in ultralow concentrations used in the therapy of alcohol abstinence syndrome possesses anxiolytic properties [4]. Delta sleep-inducing peptide (DSIP) is a component of the endogenous stress-limiting system involved in the realization of natural anti-anxiety mechanisms [3,10].

Experimental and clinical studies demonstrated that antibodies (AB) to endogenous biologically active substances in ultralow doses can modulate and simulate their effects [5]. Here we studied the efficiency of antibodies to S100 protein (AB-S100) and DSIP (AB-DSIP) in animals with experimental anxious depression.

MATERIALS AND METHODS

Ultralow doses of polyclonal rabbit AB against S100 and DSIP were used. The mixture of AB in homeopathic dilutions of C12+C30+C200 was obtained by the method of homeopathic potentiation.

Experiments were performed on male Wistar rats aging 2.5-3 months and weighing 190-220 g. Anxious depression was induced by acute stress (unavoidable electrical shock [6]) followed by water deprivation for 1 day. Then the rats voluntarily drank test preparations or water 1 h before testing. Stressed control animals received distilled water and experimental rats received AB-S100 or AB-S100+AB-DSIP mixture (1:1, 10 ml/kg).

Experimental, control, and intact rats were examined in the elevated plus-maze (EPM, 10 behavioral parameters) [9], open field (6 behavioral parameters) [1], and tail suspension tests. Each group included 10 animals. The tests were performed on different animals.

The tail suspension test and Porsolt test are routinely used for evaluation of antidepressive properties of preparations [7,8]. The latency of immobility demonstrated by rodents in the tail suspension test reflects the degree of anxiety/despair. This procedure is technically simple and more sensitive to the influence of some psychotropic preparations than the Porsolt test [2]. In our experiments the tail suspension test was used for evaluation of anxious depression in control and experimental rats 1 h after examination in EPM. Since in our studies this test was used for the first time and adapted to experimental conditions, the presence of depression was determined in control animals 24 and 48 h after stress. Stability of the qualitative index of behavioral despair (depressive state) at both terms allowed us to perform all tests 48 h after stress.

The significance of differences between groups was evaluated by one-way ANOVA using Statgraphics software.

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0007-4888/03/supp-0020$25.00

RESULTS

Intact and stressed rats considerably differed by their behavior in EPM, especially by the number of entries into open arms, time spent in open arms and center of EPM, and the ratio of transitions that inversely correlates with the severity of anxiety (Table 1). The number of rearing postures and peeping markedly decreased in stressed rats, which attested to suppression of exploratory activity due to the development of anxious depression. Changes in grooming were of particular interest. There are two types of grooming (characteristic of stress and comfort, respectively) [2], therefore not only duration, but also the type of grooming are important. In stressed animals we observed both shortening of grooming time and appearance of stereotypes (repeated cycles of the same washing movements).

The mixture of AB-S100 and AB-DSIP produced a considerable anxiolytic effect in EPM (compared to control rats with anxious depression). Antianxiety activity in AB-S100 was less pronounced.

Considerable prolongation of immobility in the tail suspension test in control stressed rats attested to the development of depression. Both preparations produced positive effects and decreased the time of immobility. It should be emphasized that the AB-S100+ AB-DSIP mixture was more effective than AB-S100.

The differences between intact and stressed rats in the open-field test also indicated that stress produced anxious depression in animals (Table 2). The mixture of AB-S100+AB-DSIP corrected behavioral changes (except defecation rate). Locomotor and exploratory activity increased. The total duration of grooming decreased due to a sharp increase in locomotor and exploratory activity. The "comfort" component prevailed in the pattern of grooming. The latency of the first movement tended to decrease. AB-S100 produced insignificant antianxiety effect.

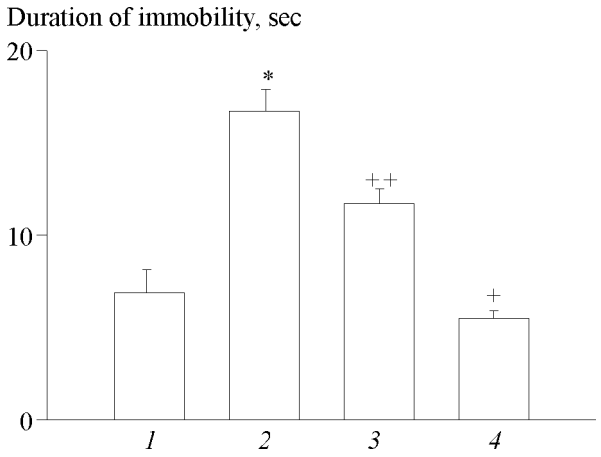

Fig 1. Effects of antibodies to S100 protein and mixture of antibodies to S100 protein and delta sleep-inducing peptide on the duration of immobility of rats with anxious depression in the tail suspension test. *1*) intact; *2*) control (depression and water); *3*) depression and antibodies to S100; *4*) depression and mixture of antibodies. **p*=0.006 compared to intact rats; ^{+}p=0.021 and ^{++}p=0.04 compared to the control.

Our results indicate that the test preparations containing potentiated AB produced an anxiolytic effect even after single administration. This probably determines the antiabstinence effect of Proproten. Interestingly, AB-DSIP potentiated the antianxiety effect of AB-S100. Studies in the open-field test suggest that AB-DSIP modulate pharmacological activity of the mixture. However, the efficiency of AB-DSIP was not demonstrated.

REFERENCES

1. Ya. Bures, O. Buresova, and P. Houston, *Methods and Main Experiments in Studying the Brain and Behavior* [in Russian], Moscow (1991).

TABLE 1. Effects of AB-S100 and Mixture of AB-S100 and AB-DSIP in Ultralow Doses on EPM Behavior of Rats with Anxious Depression (*M*±*m*)

Parameter	Intact	Control	AB-S100	AB-S100 and AB-DSIP
Number of entries into closed arms	6.5±0.8	2.3±0.3*	5.8±1.4^{++}	4.8±1.1^{++}
Time spent in closed arms, sec	253.3±9.1	289.9±2.3**	241.1±19.6^{++}	246.2±13.5^{+}
Number of entries into open arms	2.2±0.7	0.3±0.1***	2.2±0.6^{++}	2.1±0.7^{++}
Time spent in open arms, sec	22.0±8.4	2.0±1.1***	37.4±13.1^{+}	21.6±6.1^{+}
Ratio between transitions, %	18.7±2.5	7.2±1.7***	21.4±5.6^{++}	24.5±4.4^{+}
Time spent in the center, sec	22.7±4.4	8.8±1.9***	21.5±8.3	31.2±8.6^{++}
Number of rearing postures	13.0±3.8	4.3±1.1***	9.3±2.0^{++}	11.4±2.0^{+}
Number of peeping-out episodes	12.8±2.0	3.6±1.1**	8.8±2.4	10.9±2.6^{++}
Grooming, sec	84.9±14.3	16.4±5.7*	43.3±16.1	25.0±6.5
Number of fecal boluses	2.5±0.8	5.2±0.8***	2.3±0.8^{++}	1.8±0.5^{+}

Note. Here and in Table 2: **p*<0.001, ***p*<0.01, and ****p*<0.05 compared to intact rats; ^{+}p<0.01 and ^{++}p<0.05 compared to the control.

TABLE 2. Effects of AB-S100 and AB-S100+AB-DSIP Mixture on Open-Field Behavior of Rats after Stress Produced by Unavoidable Electrocutaneous Stimulation (*M*±*m*)

Parameter	Intact	Control	AB-S100	AB-S100 and AB-DSIP
Latency of the first movement, sec	4.0±0.6	6.0±1.3	4.3±0.7	3.7±0.5
Number of crossed squares	63.5±7.6	32.2±5.8**	38±7.6	69.8±9.6+
Number of central rearing postures	4.9±1.2	1.2±0.4***	1.3±0.5	5.3±1.2+
Number of peripheral rearing postures	17.8±2.0	8.8±1.8**	11.8±2.2	15.3±1.4++
Grooming, sec	25.2±4.4	37.1±6.0	20.1±6.4	15.1±3.0+
Number of fecal boluses	4.0±0.7	3.4±0.8	3.7±0.9	3.3±0.6

2. A. V. Kaluev, *Problems in Studies of Stress Behavior* [in Russian], Kiev (1999).
3. I. G. Karmanova, V. F. Maksimchuk, I. B. Voronov, *et al*., *Zh. Evolyuts. Biokhim. Fiziol*., **15**, No. 5, 583-589 (1979).
4. M. B. Shtark, T. M. Vorob'eva, O. I. Epshtein, *et al*., *Sib. Vestn. Psikhiatr. Narkol. Tomsk*, **3**, No. 21, 111-113 (2001).
5. O. I. Epshtein, V. V. Zhdanov, L. A. Stavrova, *et al*., *Byull. Exp. Biol. Med*., Suppl. **3**, 40-42 (2001).
6. C. Ferretti, M. Blengio, S. Gamalero, *et al*., *Eur. J. Pharmacol*., **280**, No. 1, 19-26 (1995).
7. A. J. Greenshaw, T. V. Nguyen, and D. J. Sanger, *Neuromethods*, Eds. A. Boulton and G. Baker, N. Y. (1988), **10**, pp. 379-427.
8. T. Onodera, R. Watanabe, K. Tha, *et al*., *Jpn. J. Pharmacol*., **83**, No. 4, 312-318 (2000).
9. S. Pellow, S. E. File, *Pharmacol. Biochem. Behav*., **24**, No. 3, 525-529 (1986).
10. D. Schneider-Helmert and G. A. Schoenenberger, *Neuropsychobiol*., **9**, No. 4, 197-206 (1983).