Involvement of the GABA-B System in the Mechanism of Action of Ultralow-Dose Antibodies to S-100 Protein

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The involvement of the GABA-B neurotransmitter system in the realization of anxiolytic and antidepressant activities of ultralow-dose antibodies to S-100 protein is demonstrated. Simultaneous injection of ultralow-dose antibodies to S-100 protein and GABA-B receptor agonist baclofen reduced the anxiolytic and antidepressant effects of the drug, while GABA-B receptor antagonist faclofen stimulated the anxiolytic and reduced the antidepressant effect of ultralow-dose antibodies to S-100 protein. The effect of ultralow-dose antibodies to S-100 protein on the GABA-B-ergic system differs from that of benzodiazepine anxiolytics (diazepam) and tricyclic antidepressants (amitryptiline) not affecting this transmitter system.

Key Words: *anxiety; depression; GABAergic system; antibodies to S-100 proteins; ultralow doses*

The GABA-ergic system is involved in the development of anxious states, while the mechanism of anxiolytic activity of benzodiazepines is linked with their GABA-A-mimetic effect [9,10,12]. However, the GABA-B system is also involved in the pathogenesis of anxiety and depression, and some GABA-B-ergic drugs exhibit antidepressant and anxiolytic properties [3,6,13,14].

It was previously shown that GABA-A-ergic system is involved in the realization of the anxiolytic effect of ultralow-dose antibodies (UDA) to S-100 protein (UDA anti-S100) [1]. Similarly as the effects of benzodiazepine anxiolytics (*e.g.*, diazepam), the anxiolytic effect of UDA anti-S-100 significantly decreased after blockade of GABA-A receptors with bicuculline and blockade of its chlorine channel with picrotoxin.

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We studied the role of the GABA-B-ergic system in the realization of anxiolytic and antidepressant effects of UDA anti-S100.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (200-250 g). The animals were divided into 8 group (10 per group) and received the following treatment: 1) (control) 2.5 ml/kg distilled water intragastrically; 2) intragastric UDA anti-S100 (2.5 ml/kg); 3) intragastric diazepam (Polfa) (2 mg/kg); 4) intragastric amitryptiline (Spofa) (15 mg/kg); 5) intraperitoneal baclofen (ICN) (1 mg/kg) and after 10 min UDA anti-S100; 6) intraperitoneal faclofen (ICN, 10 mg/kg) and after 10 min UDA anti-S100; 7) baclofen (1 mg/kg) and after 10 min diazepam (2 mg/kg); and 8) baclofen (1 mg/kg) and after 10 min amitryptiline (15 mg/kg). The anxiolytic and antidepressant activities of the drugs were studied 20 min after their administration.

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Anxiolytic effects were evaluated in a Vogel conflict test (modeled conflict between drinking motivation and painful electrical stimulation) [15]. The experiment was carried out over 3 days. During the first 24 h, the animals were deprived of water. After 24-h water deprivation the rats were trained to drink from a bottle placed in an experimental cage (275×275×450 mm). The electrode floor of the experimental cage was made of stainless steel rods (4 mm in diameter, with 8-10 mm spaces between the rods) [4]; the floor and the nipple of the bottle were connected to an electronic block.

On day 3, the animals were again placed into the experimental cage for 10 min. Ten seconds after the first water lick, constant electric current of 0.25 mA was switched on and hence, each water lick became punished, and in order to satisfy their thirst, the rats had to control the fear, which developed as a result of punishment. The number of punished water licks served as the measure of the anxiolytic effect of the drug.

Antidepressant activity of the drugs was evaluated by the method of forced swimming in a tank with wheels, used for studies of antidepressant activity [5,11]. A tank (64×30×42 cm) was divided into 4 equal sections. Wheels (11 cm wide, 10 cm outer diameter) with 2-cm paddles were placed in each section. The number of wheel rotations was recorded using magnets fixed to the edges of each wheel and hercones fixed above the wheels. The tank was filled with water (25°C) to one-half of the wheel height. The rats are placed into each section with their muzzles turned from the wheel and wheel rotation was recorded over 10 min using electromechanical counters; the increase in the number of wheel rotations indicates antidepressant activity of the drug.

Statistical processing included calculation of the means (M) and standard deviations (m) for each group. The significance of differences between the groups was evaluated using Student's t test.

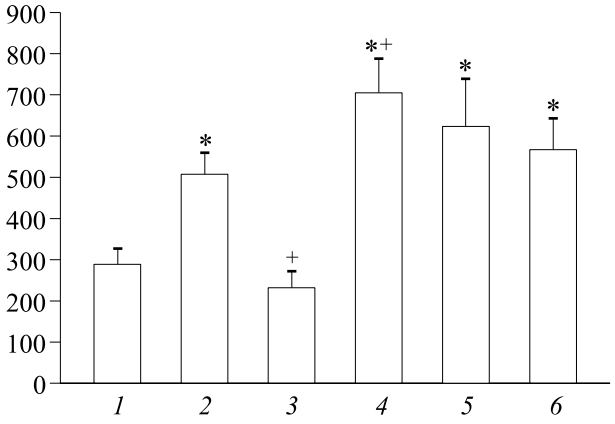

Fig. 1. Number of punished water licks in a conflict situation in rats under the effects of UDA anti-S100 and GABA-B-ergic substances. *1*) control; *2*) UDA anti-S100 (2.5 ml/kg); *3*) UDA anti-S100+baclofen (1 mg/kg); *4*) UDA anti-S100+faclofen (10 mg/kg); *5*) diazepam (2 mg/kg); *6*) diazepam+baclofen (1 mg/kg). Here and in Fig. 2: $p<0.05$ compared to: *control; +UDA anti-S100 alone.

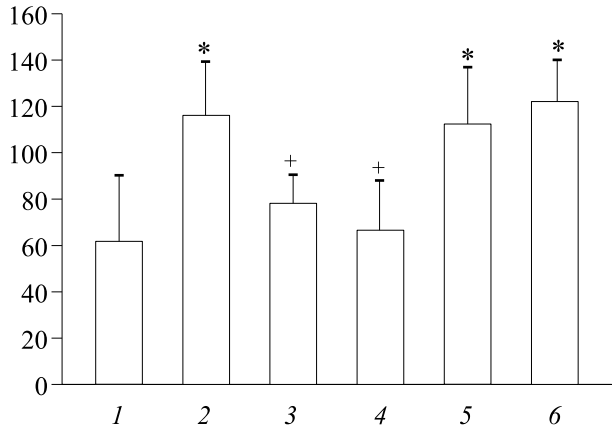

Fig. 2. Number of wheel rotations in forced swimming test in rats under the effects of UDA anti-S100 and GABA-B-ergic substances. *1*) control; *2*) UDA anti-S100 (2.5 ml/kg); *3*) UDA anti-S100+baclofen (1 mg/kg); *4*) UDA anti-S100+faclofen (10 mg/kg); *5*) amitryptiline (15 mg/kg); *6*) amitryptiline+baclofen (1 mg/kg).

RESULTS

The study confirmed previously detected anxiolytic and antidepressant effects of UDA anti-S100 [2,8]. The drug 1.8-fold increased the number of punished water licks compared to the control group ($p<0.05$; Fig. 1) in the Vogel conflict test [15] and 1.8-fold increased the number of wheel rotations ($p<0.05$; Fig. 2) in the forced swimming test. The anxiolytic and antidepressant effects of UDA anti-S100 were not inferior to those of diazepam and amitryptiline.

Baclofen (GABA-B receptor agonist) reduced the anxiolytic effect of UDA anti-S100 (2.2 times; $p<0.05$) and to a lesser extent of diazepam (1.1 times; Fig. 1). Faclofen (GABA-B receptor antagonist) potentiated the anxiolytic activity of UDA anti-S100 (1.4 times; $p<0.05$; Fig. 1).

Baclofen and faclofen similarly modified the antidepressant effect of UDA anti-S100 reducing it by 1.5 and 1.7 times, respectively ($p<0.05$; Fig. 2). Baclofen did not change amitryptiline-induced increase in the number of wheel rotations (Fig. 2).

Hence, GABA-B-ergic substances modify the anxiolytic and antidepressant activities of UDA anti-S100. Metabotropic synapses with GABA-B type receptors are located in the CNS predominantly on the presynaptic terminals (they are autoreceptors) and regulate the release of the inhibitory transmitter (GABA) [7]. The decrease in the anticonflict and antidepressant effects of UDA anti-

S100 after their simultaneous injection with GABA-B agonist baclofen seems to be a result of inhibition of UDA anti-S100-induced release of GABA. The effect of faclofen (GABA-B antagonist) on anxiolytic effect of UDA anti-S100 is opposite to that of baclofen and similar to baclofen effect on the antidepressant activity of UDA anti-S100.

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