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

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The involvement of the serotoninergic system in the realization of anxiolytic and antidepressant activities of antibodies to S-100 protein in ultralow doses is proven. Administration of ultralow-dose antibodies to S-100 protein in combination with serotoninergic agents (ketanserin and 5-hydroxytryptophan) reduced the anxiolytic and antidepressant effects of antibodies.

Key Words: *anxiety; depression; serotoninergic system; ultralow-dose antibodies to S-100 protein*

The serotoninergic (5-HT) system is involved in the genesis of anxiety and depression. The anxiolytic effect is exhibited by 5-HT1A receptor agonists (buspirone, gepirone, ipsapirone, flesinoxan) and 5-HT3 receptor antagonists (ondansetron, tropisetron, bemesetron, granisetron). Depressions are associated with 5-HT system deficiency [3,6]. Anxiety and depression are treated by 5-HT reuptake inhibitors, but other approaches to modulation of 5-HT system are now investigated, including modulation at the receptor level [7]. The anxiolytic and antidepressant activities of preparation containing ultralow-dose antibodies to S-100 protein (ULD anti-S100) were demonstrated [1].

We studied the involvement of 5-HT system in the mechanism of ULD anti-S100 action. The preparation was used in combination with 5-HT agents ketanserin (5-HT2/5-HT1C receptor blocker [8,11]) and 5-HT precursor 5-hydroxytryptophan (5-HTP).

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (200-250 g). The animals were divided into the following groups: 1) controls (2.5 ml/kg distilled water intragastrically); 2) 2.5 ml/kg ULD anti-S100 intragastrically; 3) 2 mg/kg diazepam (Polfa) or 15 mg/kg amitryptiline (Spofa) intragastrically; 4) 1 mg/kg ketanserin (ICN) intraperitoneally; 5) 50 mg/kg 5-HTP intraperitoneally (ICN); 6) ketanserin and after 10 min ULD anti-S100; and 7) 5-HTP and after 10 min ULD anti-S100. Anxiolytic and antidepressant activities were evaluated 20 min after drug administration.

Anxiolytic effects of the drugs were evaluated in a conflict situation (conflict between drinking motivation and painful electric stimulation) [9]. Experiment was carried out over 3 days. On day 1 the animals were completely deprived of water. After 24-h deprivation the rats were trained to drink water from a drinking bottle placed in the experimental box. The box (275×275×450 mm) had a standard electrode floor from stainless steel rods (4 mm in diameter, 8-10 mm distance between rods)

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[2]. The electrode floor and the bottle nipple were connected to a power module.

On day 3, the animals were again placed into the experimental box for 10 min. Direct current (0.25 mA) was delivered to the floor and bottle nipple 10 sec after the first lick. Hence, each lick became punished, and in order to satisfy their thirst, the rats had to overcome the fear resultant from punishment. The number of punished licks was considered as a measure of the drug anxiolytic effect intensity.

Antidepressant effect was evaluated by forced swimming in a reservoir with wheels, which, along with Porsolt's method, is widely used in studies of antidepressants [4,8]. The device consisted of a reservoir (64×30×42 cm) divided into 4 equal sections with 11-cm-wide paddle wheels with 12 paddles 2-cm wide, so that the external diameter of the wheels were 10 cm. Magnets were fixed at the edge of each wheel; hercones working each time when the magnets passed below were fixed above the wheels. The tank was filled with water (25°C) by half of the wheel's height. A rat was placed into a section with its back to the wheel and the number of wheel revolutions was recorded for 10 min with electromechanical counters (increase in the number of wheel revolutions indicated antidepressant activity of the drug).

The significance of differences between the groups was evaluated using Student's *t* test.

RESULTS

The ULD anti-S100 preparation exhibited a pronounced anxiolytic effect in the conflict situation test: the number of punished licks increased significantly in comparison with the control (Table 1). This effect was comparable to the effect of diazepam.

Ketanserin (5-HT2/5-HT1C receptor antagonist) exhibited anxiolytic activity and increased the number of punished licks; 5-HTP also increased the number of punished licks, though negligibly (Table 1).

Treatment with ULD anti-S100 in combination with ketanserin or 5-HTP attenuated the anticonflict effect of ULD anti-S100 (Table 1).

In the forced swimming test, control rats placed into water tried to get out by using the wheels. As the wheels rotated freely, these attempts were useless and the rats abandoned them, though resumed their activity from time to time. Much more wheel revolutions were recorded in the group treated with ULD anti-S100 (Table 1), this indicating antidepressant effect of the preparation, comparable to the effect of classical antidepressant amitryptiline.

TABLE 1. Changes in Punished Drinking in a Conflict Situation and Number of Wheel Rotations in Forced Swimming Test in Rats under the Effects of ULD Anti-S100 and Serotoninergic Drugs (*M±m*)

Drug	Number of punished water gulps	Number of wheel revolutions
Control	102.2±15.06	73±25.19
ULD anti-S100	324.9±40.61*	159.5±29.77*
Diazepam	372.6±45.18*	n.d.
5-HTP	135±33.15	146.6±30.16*
Ketanserin	224.5±31.7*	117.1±24.87*
ULD anti-S100+ 5-HTP	259.2±48.4*	102.9±44.36
ULD anti-S100+ ketanserin	168.7±40.12+	80.5±18.14+
Amitryptiline	N.d.	119.1±19.16*

Note. $p<0.05$ compared to: *control, +ULD anti-S100. N.d.: not determined.

Ketanserin and 5-HTP monotherapy increased the number of wheel revolutions, thus exhibiting antidepressant activity. Their combination with ULD anti-S100 reduced the antidepressant effect of the preparation, this reduction being significant in ketanserin+ULD anti-S100 in comparison the effect of ketanserin alone (Table 1).

Hence, ULD anti-S100 are not inferior to diazepam and amitryptiline by anxiolytic and antidepressant activities, respectively. The decrease in anxiolytic and antidepressant effects of ULD anti-S100 in combination with ketanserin and 5-HTP indicates the involvement of 5-HT system in the realization of the effects of ULD anti-S100. The pattern and mechanisms underlying modulation of the effect of the test preparations deserve more detailed investigation. Importantly that the effects of ketanserin and 5-HTP are mediated by different mechanisms: anxiolytic effect of ketanserin is determined by selective blockade of 5-HT2/5-HT1C receptors, while the effect of 5-HTP is due to increased availability of 5-HT in the hippocampus and its effect on 5-HT1A receptor [6]. Hence, reduction of the activities of both substances (ketanserin and 5-HTP) with ULD anti-S100 does not contradict the notions on the 5-HT system.

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