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Original Article

Improvement of Memory by Means of Ultra-Low Doses of Antibodies to S-100B Antigen

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Antigen S-100B of nervous tissue, according to the data of numerous studies, affects the mechanisms of nervous system plasticity and memory. The influence of ultralow doses of antibodies to S-100B (6C dilution, according to the homeopathic pharmacopoeia) has been studied on three learning behavioral models on Wistar rats, which were inhibitory avoidance, choosing of bowls with sucrose and feeding behavior cessation after auditory signal. For all three tasks, parameters of reproduction of the learned skills improved after per oral administration of potentiated antibodies to S-100B antigen immediately after learning. Possible mechanisms of the anti-S-100B antibodies influence on memory formation are discussed.

Keywords: Antigen S-100B – antibodies – ultra-low doses, memory

Introduction

There is a constant interest in modern neuropharmacology and medicine for analyzing physiological activity of low and ultralow doses of drugs obtained by homeopathic methods (1–6). A clear advantage of these drugs is absence of direct toxic effects that makes possible their wide use. Preparations of antibodies to neurospecific antigens are of high interest among such drugs, as such antigens are involved in regulation of basic functions of the nervous system. It also relates to the antigen S-100B of nervous tissue that controls neuroglial relationship and, according to data of numerous studies, affects mechanisms of plasticity of the nervous system (7–15).

At the Institute of Molecular Biology and Biophysics, the effects of antibodies to brain-specific antigen S-100 B on the mechanisms of plasticity of the nervous system, including the post-tetanic potentiation, have been studied for years (16). A new stage began to analyze low concentrations of such antibodies that often have an opposite effect to that of high doses (3,4). The present work is a continuation of these studies.

The purpose of the work was to study the effects of ultralow doses of anti-S-100B antibodies (preparation 'Proprotene-100') on memory formation in rats. Three various types of tasks were used as learning models: (i) inhibition of animal's descent from a safe platform onto an electrified grid, (ii) choosing a bowl with sucrose solution and (iii) inhibition of feeding behavior after auditory signal.

Methods

Antibodies

To prepare potentiated antibodies rabbit monospecific multivalent serum to S-100B was used. Antibodies to S-100B were isolated from the serum on columns with S-100B protein immobilized on CNBr-sepharose (17,18). The immunoglobulin solution was dialyzed against 0.15 M NaCl and concentrated using ultrafiltration. Antibodies did not affect liver, lungs, kidney and other organs. Ultralow concentrations of the antibody were obtained using routine homeopathic methods in 'Materia Medica Holding' company (Research and Production Company, Moscow). The solution of antibodies (12 mg ml^{-1}) was mixed with lactose in a ratio of 1:100 (0.05 ml of solution, 5 g of lactose) that made dilution C1. Dilution C2 was obtained by adding 9.9 g lactose to 0.1 g C1. Distilled water was added in a ratio of 1:99 (0.25 g/24.75 g). Then it was stirred no less than 10 times to make the dilution C3. Dilution C4 was

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obtained by adding 50% ethanol solution (0.25 g/24.75 g). C5 and then C6 were prepared using addition of distilled water and mechanical stirring. Potentiated water was prepared using water instead of antibody solution. 6C dilution (ratio $1:10^{12}$) was used in the experiments.

Rats

Experiments were performed on adult male Wistar rats weighing 200–300 g from the animal house of the Novosibirsk National Academy of Medical Sciences. Rats were kept in pairs with a free access to water and food, under a 12 h illumination per day. Solution of potentiated antibodies to S-100B was administered to rats *per os* (0.5 ml) immediately after three tasks learning sessions described above. During learning to respond to auditory stimulus, solution was additionally administered 24 and 1 h before training. Control animals were given potentiated water (0.5 ml). During learning to choose a bowl with sucrose, dexametasone was administered *per os* to an additional comparison group in a dose of 0.3 mg kg^{-1}, which influence memory formation (19,20) due to affecting amygdale, which is one of the key structures of brain functioning (21,22).

Development and Testing of Inhibitory Avoidance

Training of inhibitory avoidance was carried out in a veneer box of 50 × 25 × 25 cm. The floor in the testing chamber consisted of parallel bronze bars with a diameter of 3 mm, the distance between bars being 1 cm. On the left a 7 cm wide and 2.5 cm high wooden platform was located on which an animal was delicately placed (23). During learning avoidance reaction, the animal was descended from the platform, and when all the four paws were on the grid, electric current was automatically switched on (0.5 mA, 50 Hz, 2 s). The animals were tested in 24 h time, with the electric current switched off. Learning session was repeated in 4 weeks, conservation of the inhibitory avoidance was tested in 24 h and in 7 days. Time of the rat's descent from the platform was recorded; maximal duration of the test did not exceed 5 min.

Learning to Choose Sucrose Bowls

Experiments on choosing bowls with sucrose solution were carried out in the testing chamber made of organic glass (40 × 20 × 20 cm), and a floor made of metal plates. At each side of the chamber 4 cm above the floor there were two bowls, each 3 cm in diameter, the distance between the bowls was 5 cm. The bowls contained 20% sucrose solution. The animals were previously kept in the chamber for 10 min for four consecutive days. Those with low consumption of sucrose (<5% of exposition time) were excluded from the experiment. During training session, the bowls on the left were put under electric current of 0.15 mA, 50 Hz with a latent period of 0.1 s. Learning sessions lasted 15 min and were repeated in 2 days. In experiments numbers of animals' contacts with the bowls separated by no less than 3 s intervals were estimated.

Avoidance Reaction by Auditory Signal

Experiments were carried out in a chamber similar to those described above, with bowls containing sucrose, and an auditory signal. Animals were previously kept in the chamber for 10 min for four consecutive days. Those with low consumption of sucrose (<5% of exposition time) were excluded from the experiment. During learning sessions, with the beginning of animal's consumption of sucrose solution the following method was used: in 7 s time a sound of 800 Hz, 20 dB was switched on. Three seconds later all the bowls were under electric current of 0.15 mA, 60 Hz. Combined action of the stimuli lasted for 5 s. The session lasted 20 min and included 10 auditory signals that animal could obtain. Testing was made under switched-off current in 24 h and in 7 days. Testing lasted 20 min. Estimated were latent periods of cessation of drinking behavior after its start, completion of drinking with sound signal and resuming of drinking behavior after cessation of sound.

Stimulation and Registration of Reactions of Rats Were Carried Out Automatically Using a Computer

Statistics

Results were expressed as the mean ± SEM. Comparison of data among three groups was performed using the one-way analysis of variance (ANOVA) with Bonferroni's post-test. Comparison of data between two groups was performed by Student's test based on the variance of data examined by *F*-test. When the *P*-value was <0.05, the difference was considered to be significant.

Results

Ultralow Doses of Antibodies to S-100B and Inhibitory Avoidance

In the experiment on development of inhibitory avoidance, the latent period of descent from the platform in control and experimental groups was about 4 s (see Table 1). When tested in 24 h, the differences between the groups were not significant. After 4 weeks the animals were trained repeatedly. Testing of the reaction reproduction in 24 h indicated significant increase of time of staying on the safe platform ($P < 0.05$) of animals of the experimental group versus the control group (by 44%). The next testing in 7 days showed a conservation of the difference between experimental groups of animals. The rats that were administered with antibodies stayed on the platform 254 s on average, and those that were given water stayed 144 s ($P < 0.01$).

Table 1. Latencies of rats' descent from the platform

Group of animals	Control, $n = 14$	Antibodies 6c, $n = 14$
Learning		
Learning	4.2 ± 1.1	4.1 ± 1.8
Test in 24 h	28.7 ± 10.4	49.2 ± 23.2
Repeated learning		
Learning	40.5 ± 21.0	64.6 ± 29.4
Test in 24 h	188.2 ± 34.2	272.4 ± 19.4*
Test in 7 days	144.5 ± 25.9	254.4 ± 10.7**

*$P < 0.05$, **$P < 0.01$ versus control.

Table 2. Time courses of animals' attempts to consume sucrose from the right-hand drinking bowls

Groups of animals	Control, $n = 8$	Antibodies, $n = 8$	Dexamethasone, $n = 7$
Prior to learning	11.75 ± 3.03	10.35 ± 2.49	12.71 ± 1.56
Sessions 1 + 2	14.50 ± 3.13	11.50 ± 3.47	7.85 ± 1.94
Sessions 3 + 4	14.62 ± 2.88	13.00 ± 4.45	8.42 ± 3.25
Sessions 5 + 6	19.62 ± 5.45	19.75 ± 5.25	12.85 ± 3.40

Table 3. Time courses of animals' attempts to consume sucrose from the left-hand drinking bowls

Groups of animals	Control, $n = 8$	Antibodies, $n = 8$	Dexamethasone, $n = 7$
Prior to learning	12.62 ± 3.11	10.75 ± 2.75	13.28 ± 1.68
Sessions 1 + 2	12.37 ± 2.51	7.62 ± 1.64	7.14 ± 1.03
Sessions 3 + 4	11.62 ± 2.40	6.12 ± 1.35	4.28 ± 1.84*
Sessions 5 + 6	9.75 ± 1.94	4.75 ± 0.94*	4.00 ± 0.69*

*$P < 0.05$ versus control.

Ultralow Doses of Antibodies to S-100B and Reaction of Choosing Bowls with Sucrose Solution

Analysis of dynamics of learning to choose 'safe' bowl with sucrose solution demonstrated that there were no considerable differences in the number of contacts with bowls on the right side (no punishment) between control and experimental, experimental and dexamethasone groups, although in the former two groups there was a strong tendency to increase the number of such contacts from 10–11 to 19–20 as the training went on, whereas in animals administered with dexamethasone there were no changes (see Table 2).

Different dynamics were registered for bowls on the left side with 'punishment' (see Table 3). As early as 3rd–4th session (the data of each two consecutive trainings were pooled), the rat group that were given dexamethasone showed a significant decrease of numbers of contacts as compared with the control (11.6 and 4.2), whereas in the group that was given potentiated antibodies there was only a tendency to 6.1. By the 5th–6th training sessions, the decrease in number of contacts with bowls on the left side as compared to the control was significant ($P < 0.05$) in the two experimental groups (control, 9.7; antibodies, 4.7; dexamethasone, 4.0 contacts).

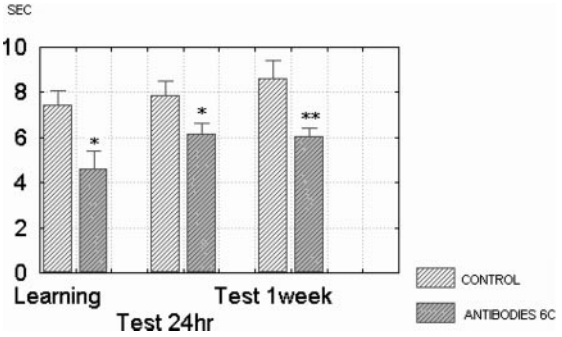

Figure 1. Behavioral characteristics during development of the avoidance reaction to an acoustic stimulus: latency of avoidance (seconds). *Note*: *$P < 0.05$ versus control, **$P < 0.01$ versus control. There were 10 animals in each group.

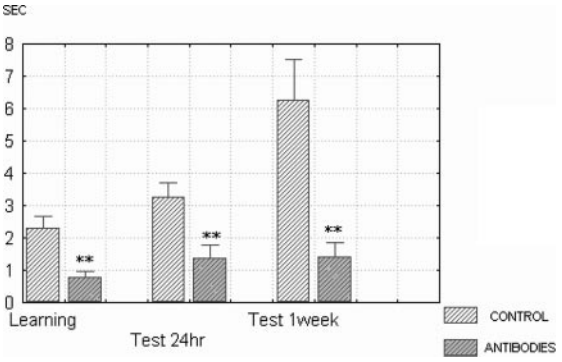

Figure 2. Behavioral characteristics during development of the avoidance reaction to an acoustic stimulus: duration of sucrose consumption on background of a sound stimulus (seconds). *Note*: **$P < 0.01$ versus control. There were 10 animals in each group.

Ultralow Doses of Anti-S-100B Antibodies and Avoidance Reaction to Auditory Signal

In the development of avoidance reaction to auditory signal, the operational conditioning reflex developed after two or three combinations, so that conditioned reactions appeared as soon as after 10 exposures to the stimulus. The animals that had been previously administered antibodies to S-100B antigen showed a higher speed of learning than the control, which was indicated as significant reduction of avoidance latency (7.42 and 4.59 s, $P < 0.05$, see Fig. 1) and decrease of time of consuming sucrose solution when exposed to auditory stimulus (2.28 and 0.78 s, $P < 0.01$, see Fig. 2).

In 24 h after learning session, the avoidance latency remained reduced in animals that had been administered with antibodies as compared to the control (7.86 and 6.17 s, $P < 0.05$, Fig. 1). Time of sucrose consumption on the background of the auditory stimulus also decreased (3.27 and 1.37 s, $P < 0.01$, see Fig. 2).

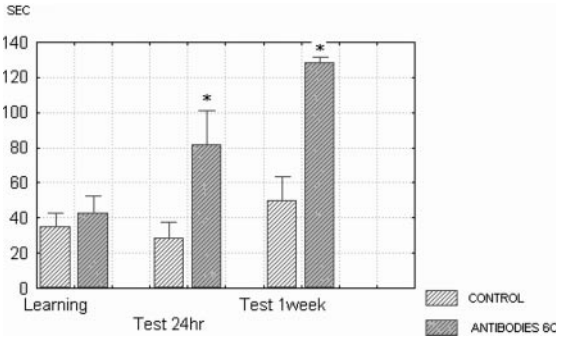

Figure 3. Behavioral characteristics during development of the avoidance reaction to an acoustic stimulus: renewal of drinking behavior in seconds. $*P < 0.05$. *Note*: $*P < 0.05$ versus control. There were 10 animals in each group.

In a week after learning, the above changes persisted. For the avoidance latency control group had 8.62 s, experimental group showed 6.05 s, $P < 0.01$; for sucrose consumption on the background of sound signal control group showed 6.26 s, and experimental group showed 1.40 s, $P < 0.01$ (Figs 1 and 2).

It is noteworthy that during the development of a conditioned reflex, the acquisition of signal significance by sound seems to have been accompanied by development of conditioned reaction to time, which affected behavioral parameters and resulted in manifestation of avoidance before the effect of the auditory stimulus. Nevertheless, effects associated with sound turned out to be the most demonstrative: in the control group duration of sucrose consumption on the background of sound signal increased from the first to the second testing ($P < 0.05$), i.e. a decrease of conditioned inhibition was shown. This may be characterized as a manifestation of extinction in the control group and a higher reflex stability in the experimental group that has not shown such an increase (see Fig. 2).

Apart from the mentioned differences between groups of animals related to avoidance latencies, in 24 h and in a week after training, intervals of the resumption of sucrose solution consumption increased in animals that had been administered antibody preparations (49.97 and 128.09 s, $P < 0.05$; Fig. 3).

Discussion

The rats that were administered antibodies on the average stayed on the platform longer than rats that were given water. These results confirm better memorizing aversive stimulation under the influence of low doses of antibodies to S-100B. Administration of both dexamethasone and potentiated antibodies improved the avoidance of 'dangerous' bowls. The animals preliminarily administered antibodies to S-100B antigen showed faster learning than the control group, which was expressed in a significant reduction of avoidance latency in the auditory task. The effects of conditioned stimulus expressed as the rate of development and duration of suppression of the feeding behavior were stronger in experimental than in control animals.

Animals in the present study were exposed to three types of learning tasks. First one required inhibition of motor activity, the second required choosing one of two objects and the third involved learning a skill to stop feeding behavior by auditory signal. The tasks included processing of information obtained through various sensory channels, proprioceptive, visual and auditory. In all three cases, administration of potentiated antibodies to S-100B improved learning skills and facilitated long-term and short-term (working) memory formation in the task of avoiding of auditory signal.

When discussing possible mechanisms of effects of potentiated antibodies on memory, it is expedient to remember that the amount of S-100B protein in the brain increases during learning and the anti-S-100 antibodies in usual doses disturb the processes of memory consolidation (11,24). The influence of S-100B on memory seems to be associated with the regulation of transcription process by these proteins (12,14). Therefore, the effect of S-100B is associated with memory improvement.

The effect of antibodies to S-100B in ultralow doses may be directly opposite to that of usual doses of antibodies, therefore improving the learned skills reproduction (25). It was demonstrated in the present work as well that Anti-S-100 antibodies in usual doses changed the frequency of action potential generation in spontaneously active neurons and blocked formation of long-term potentiation (model of memory) in mossy fiber synapses, but 20 min pre-incubation of snail ganglia or hippocampal slices with ultralow dose (6C) anti-S-100 abolished the effects of the same antibodies in high concentrations (3).

The following explanations of this situation are possible. First, usual doses of antibodies decrease the functional activity of S-100B antigen molecules, inhibits the electrical activity of neurons (26) and the stimulation of adenylate cyclase (27), whereas its ultralow doses enhance the functional activity of the protein, e.g. by changing of S-100B binding with neuron and glial membranes (16). Second, low doses of antibodies via regulatory mechanisms enhance the release of S-100B molecules from glial cells, changing membrane electric potential. There are receptors of 5-HT on glial cells. Stimulation of these receptors enhanced the release of S-100B from these cells (9,28). It may be regarded as one of the mechanisms of modulation memory via serotonergic system (29). Ultralow doses of anti-S-100B antibodies probably may affect in similar way, enhancing the release of S-100B. All this ultimately can result in a more efficient influence of S-100B on the transcription of genes (12,14) involved, as it is believed, in the memory trace formation.

The effect of ultralow doses of antibodies to S-100B on the mechanisms of brain plasticity is determined presumably by their interaction to the antigen on the outer surface of neuronal membranes and glial cells, by influencing its release via glial cells or penetration into neurons. Finally, the ultralow doses of anti-S-100B may directly affect autoantibodies (3), possibly involving physical–chemical properties and structure of water solution (30). Memory disorders in psychiatric patients are

characterized by high levels of autoantibodies to S-100 protein (31,32). Thus, yet another possibility of getting control over memory mechanisms of men and animals is shown. At present, the authors of the paper are in the process of studying the influence of ultralow doses of antibodies to S-100B in the experiments on animals with learning and memory disorders, e.g. rats genetically predisposed to catalepsy (33).

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