Effects of Antibodies against S-100 Antigen in Ultralow Doses (Proproten-100) on Acquisition of Avoidance Response in Rats

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We studied the effect of potentiated antibodies against S-100 antigen on learning of avoidance responses of 2 types. Peroral administration of antibodies promoted inhibition of locomotor activity and feeding behavior, which was associated with electrical pain stimulation. Our results indicate that the preparation in ultralow doses modulates the mechanism of memory formation.

Key Words: *avoidance response; ultralow doses; antibodies; S-100 antigen; memory engram*

Physiological activity of potentiated preparations in low and ultralow doses is widely studied in neuropharmacological experiments [2]. These preparations have no undesired properties and can be extensively used in clinical practice [3]. Preparations of antibodies against nerve-specific antigens involved in the regulation of the major functions of the nervous system attract much attention. S-100 antigen expressed in the nervous tissue regulates the neuroglial relationships and plays a role in the mechanisms of learning and memory [5,6,8]. Previous studies showed that antibodies against S-100 antigen in ultralow doses modulate conditioned activity of animals [4].

Much attention is paid to nootropic preparations that prevent brain dysfunction in damage or disease (*e.g.*, Alzheimer's disease) leading to severe memory disorders. The preparations modulating learning are of considerable interest in this respect.

Here we studied the effect of antibodies against S-100 antigen in ultralow doses (Proproten-100) on memory formation in rats. Two types of the avoidance response were used as the models of learning: inhibition of locomotor activity in a shuttle box and suppression of feeding behavior by simultaneous acoustic and electrocutaneous stimulation.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=40) weighing 200-280 g and obtained from the nursery of the Novosibirsk State Medical Academy. The animals were housed in cages (2 rats per cage) under natural light/dark regimen and had free access to water and food. The rats were randomly divided into 2 experimental and 2 control groups.

The solution of potentiated antibodies against S-100 antigen (C12+C30+C200, Materia Medica Holding) was mixed with water in drinking bowls (25% of total volume, daily dose 4.2±0.2 ml). It was given 1 day before training and removed after 48 h. Controls received the corresponding solvent.

The rats were trained to reduce the intensity of illumination in a shuttle box [1] by transition form one compartment to another. The avoidance response was conditioned by delivering electrocutaneous stimulus (0.5 mA, 1 sec) after animals reaction to light. The response was tested 1 day and 1 week after learning. Six light stimuli (40 W, 40 sec) were delivered at 40-sec intervals. Locomotor activity was evaluated by

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TABLE 1. Behavioral Parameters during Passive Avoidance Conditioning (*M*±*m*)

Parameter		Before learning	After learning	
			after 1 day	after 1 week
Locomotor activity	control	13.00±1.41	5.87±1.73	6.87±2.03
	treatment	11.25±1.45	1.12±0.26*	4.12±1.37
Latency of reaction to light stimulation, sec	control	25.27±2.80	32.31±2.74	29.85±3.53
	treatment	26.15±2.67	39.11±0.58*	33.21±2.72

Note. *$p<0.05$ compared to the control.

the number of transitions between two compartments and latency of switching off the lamp.

Avoidance response to acoustic stimulation was elicited in a transparent chamber (32×18×20 cm). Drinking bowls were filled with 20% sucrose. The animals were adapted to experimental conditions for several days. In the experimental session, intermittent sound (800 Hz) was delivered 7 sec after the start of drinking. Electric current (150 mA, 50 Hz) was applied to the drinking bowl through a metal floor 3 sec after the start of acoustic stimulation. The duration of simultaneous stimulation with electric current and sound was 5 sec. The avoidance response was tested without electric stimulation 24 h and 7 days after learning.

We estimated the latency of the avoidance response or mean time of drinking behavior interrupted by acoustic stimulation, mean time of sucrose consumption during conditioned stimulation, and mean interval between the end of stimulation and start of drinking behavior. These parameters reflected the inhibition of feeding behavior after learning.

The results were analyzed by Student's *t* test.

RESULTS

Before acquisition of the passive avoidance response, the control and treated rats did not differ in locomotor activity and latency of the reaction to light. Locomotor activity of animals decreased, while the response latency increased 1 day after training. Behavioral changes were most pronounced in rats receiving potentiated antibodies (Table 1). These data indicate that the preparation of antibodies improves memory about aversive stimulation.

Conditioned avoidance response to acoustic stimulation was elicited after 2-3 presentations (no more than 10 presentations). The rats pretreated with antibodies against S-100 antigen exhibited higher learning capacity compared to the control animals. It was manifested in shortened latency of the avoidance response and time of sucrose consumption during acoustic stimulation (Table 2). Intergroup differences persisted 1 day and 1 week after training.

Conditioned reaction to time probably developed during conditioning with acoustic stimulation. It modulated behavior and manifested in appearance of the avoidance response before acoustic stimulus. However, the sound-produced effect was most pronounced. The duration of sucrose consumption in control animals significantly increased in repeated tests with acoustic stimulation ($p<0.05$), which reflects the process of extinction. Treated animals did not exhibit these changes, which illustrates better memory consolidation (Table 2).

The interval to continue sucrose consumption increased in antibody-receiving rats 1 day and 1 week

TABLE 2. Behavioral Parameters during Avoidance Conditioning with Acoustic Stimulation (*M*±*m*)

Period, group		Response latency, sec	Period of sucrose consumption during acoustic stimulation, sec	Continuation of feeding behavior, sec
Training	control	7.42±0.63	2.28±0.40	35.11±7.99
	treatment	4.59±0.79*	0.78±0.19**	42.81±9.49
After 1 days	control	7.86±0.63	3.27±0.44	28.32±9.27
	treatment	6.17±0.45*	1.37±0.39**	81.97±19.02*
After 7 days	control	8.62±0.79	6.26±1.25	49.97±13.37
	treatment	6.05±0.38**	1.40±0.44**	128.09±33.74*

Note. *$p<0.05$ and **$p<0.01$ compared to the control.

after training (Table 2). These results indicate that the effect of conditioned stimulation in treated rats (period of acquisition and inhibition of feeding behavior) was more potent than in control animals.

Intergroup differences in the degree or duration of suppression of feeding behavior were probably related to the influence of potentiated antibodies on "memory incubation" [7]. Probably, this preparation modifies the effect of S-100 protein on gene expression and nerve tissue growth during learning [5,6,8].

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