Effect of Ultralow Doses of Antibodies to S-100 Antigen (Proproten-100) on Spatial Learning in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 2, pp. 163-165, February, 2005
Original article submitted August 23, 2004

In experiments on rats we studied the effect of potentiated antibodies against S-100 antigen on training a step-down passive avoidance task and choice between drinking bowls with sucrose solution. Peroral treatment with antibodies accelerated inhibition of ineffective and punished locomotor reactions in animals.

Key Words: *spatial learning; ultralow doses; antibodies; S-100 antigen; memory engram*

S-100 antigen is a brain-specific protein that regulates neuroglial relationships and plays a role in the mechanisms of learning and memory [2,3]. Previous studies showed that antibodies to S-100 antigen in ultralow doses (Proproten-100) modulate avoidance learning in rats [1]. A combination of light or acoustic stimuli with electrocutaneous stimulation inhibited stereotypic reactions in animals, including locomotion in a shuttle box and sucrose drinking. Peroral administration of antibodies potentiated the inhibitory effect of these stimuli.

Here we studied the effect of antibodies to S-100 protein in ultralow doses on spontaneous behavior of rats associated with environmental orientation. Experiments were performed on the following models of learning: step-down passive avoidance paradigm; and the choice between the right or left drinking bowl with sucrose solution. Synthetic corticosteroid dexamethasone (DM) improving memory function served as the reference preparation in experiments with drinking bowl choice [5-7].

MATERIALS AND METHODS

Experiments were performed on 52 male Wistar rats 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 12:12-h light/dark regimen and had free access to water and food.

The step-down passive avoidance learning was performed in a 50×25×25-cm chamber. The floor of this chamber was made of bronze rods (3 mm in diameter) positioned at a distance of 1 cm from each other. The animal was placed on a platform (size 25×7 cm, height 2.5 cm) in the left side of the chamber [4]. During learning electric current was applied to the grid floor (0.5 mA, 50 Hz, 2 sec) when the rat stepped down from the safe platform on the floor with all limbs. The animals were tested without electric stimulation 1 day after training. Repeated training was performed after 4 weeks. Passive avoidance performance was tested after 1 and 7 days. The step-down latency served as a criterion of learning (300 sec for non-responding animals).

The solution of potentiated antibodies (PAB) against S-100 antigen (C6+C12+C200, Materia Medica Holding) was given perorally 30 min before (0.5 ml, series I) or immediately after training (repeated training, series II). Control animals received potentiated water.

Experiments with the choice between drinking bowls with sucrose solution were performed in an organic glass chamber (40×20×20 cm). The floor of this chamber was made of stainless steel plates. Two drinking bowls with 20% sucrose solution were fixed on each wall of the chamber at a height of 4 cm from the floor. The diameter of drinking bowls was 3 cm. The distance between drinking bowls was 5 cm. Du-

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0007-4888/05/1392-0196

TABLE 1. Step-Down Latency in Rats (*M*±*m*)

Group	Control (*n*=14)	Treatment (*n*=14)
Series I		
Training	4.2±1.1	4.1±1.8
Test after 1 day	28.7±10.4	49.2±23.2
Series II		
Training	40.5±21.0	64.6±29.4
Test after 1 day	188.2±34.2	272.4±19.4*
after 7 days	144.5±25.9	254.4±20.7**

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

ring training, electric current (0.15 mA, 50 Hz) was applied to the drinking bowl through a metal floor when the animal attempted to drink from the left bowl. The latency of stimulation was 0.1 sec. Training (15-min session) was repeated after 2 days. The number of approaches to the left and right drinking bowls (interval not less than 3 sec) was determined. The solution of PAB (0.5 ml) was given perorally immediately after training. The solution of potentiated water or DM in a dose of 0.3 mg/kg served as the control.

The results were processed using Student's *t* test and two-factor dispersion analysis.

RESULTS

Series I showed that PAB do not modulate step-down latency (4 sec), which attests to the absence of the effect of the test preparation on locomotor activity of animals (Table 1). The latency of locomotor activity in control and treated rats did not differ after 24 h. In series II the animals were repeatedly trained with electrical stimulation during step down from the platform. Testing on the next day showed that treated rats spent much longer time on a safe platform compared to control animals (Table 1). These differences were also observed after 7 days. Therefore, PAB improved memory about the aversive stimulus.

Learning of drinking from a safe bowl with sucrose showed that control, treated, and DM-treated rats do not differ by the number of contacts with the right (unpunished) bowl. It should be emphasized that the number of contacts with the right drinking bowl tended to increase in control and treated animals during training (Table 2). We revealed a decrease in the number of approaches to the left (electrified) bowl. The number of these approaches significantly decreased in DM-treated rats after 3-4 training sessions (pooled data of 2 consecutive sessions, Table 3). The differences between PAB-treated and control animals were revealed by the 5th or 6th training session.

DM in a dose of 0.3 mg/kg improves acquisition of avoidance behavior associated with step down from a safe platform to electrified floor [5]. The test preparation in this dose prevented impairment of spatial memory in a water maze produced by adrenalectomy [6,7]. Our experiments showed that DM improves spatial memory (choice between the right and left drinking bowls). It was manifested in a decrease in the number of punished reactions. PAB produced the same effect (Table 3).

Our findings suggest that PAB to S-100 protein and DM produce the same effect on avoidance learning in the step-down paradigm and choice between drinking bowls. PAB to S-100 protein accelerated the inhibition of ineffective and punished locomotor reactions in animals. These data show that the test preparation plays a role in the mechanisms of spatial memory.

TABLE 2. Number of Contacts with Right Drinking Bowl Containing Sucrose Solution (*M*±*m*)

Group	Control (*n*=8)	PAB (*n*=8)	DM (*n*=7)
Before training	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. Number of Attempts to Left Drinking Bowl with Sucrose Solution (*M*±*m*)

Group	Control (*n*=8)	PAB (*n*=8)	DM (*n*=7)
Before training	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*

Note. *p<0.05 compared to the control.

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