PHYSIOLOGY

Genetic Mechanisms of the Involvement of SMP-69 Protein in Memory Consolidation

A. A. Mekhtiev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 8, pp. 141-143, August, 1999
Original article submitted November 23, 1998

Intracerebral administration of antibodies to SMP-69 protein to rats 24 h prior to passive avoidance conditioning impaired memory processes, while the administration 48 h after learning had no such effect. Activation of RNA and protein synthesis was observed 24 h after the administration. It is suggested that the impaired consolidation of memory traces is due to the synthesis of "anticonsolidation" proteins.

Key Words: *antibodies to SMP-69 protein; memory consolidation; transcription; translation*

Studies on different models of learning have shown that serotonin is involved in the consolidation of memory traces [4,8,12,14]. Both the decrease in the brain content of serotonin after destruction of serotoninergic neurons with intracerebrally administered specific neurotoxins [2] and experimental elevation of brain serotonin [15] impair memory formation. The involvement of serotonin in memory consolidation is determined by its regulatory effect on genetic apparatus of neurons [9]. Therefore, investigation of serotonin effects on genome activity can clarify the mechanisms of its participation in memory consolidation. Previously, we identified and purified a new serotonin-modulated brain protein SMP-69 [1,5].

This study analyzed the effects of antibodies (AB) to SMP-69 on memory processes and genomic activity in rat neurons.

MATERIALS AND METHODS

Polyclonal AB to SMP-69 were isolated by affinity chromatography from the serum of rabbits immunized for 3-4 months with SMP-69 [7].

A. I. Karaev Institute of Physiology, Azerbaijan Academy of Sciences, Baku

Passive avoidance conditioning was used to assess the effect of AB on memory processes. Experimental chamber consisted of 2 compartments (illuminated and dark) connected by a narrow passage. During the acquisition procedure, the rat was placed into the light compartment and after transition into the dark compartment received intermittent footshock (0.8 mA) applied through electrified floor. After shock the animal returned to the light compartment and was taken out. In the retention test (10-min session) the animal was placed into the light compartment and was not shocked when entering the dark compartment. During this test the following indices were measured: the latency of entering the dark compartment, the time spent in each compartment and near the entrance to the dark compartment.

Preparations were administered in the left lateral ventricle under ether anesthesia. The rats were divided into experimental and control groups (n=12 each). The experimental rats received 10 μl AB (1.5 mg/ml), control rats received the same volume of nonimmune γ-globulins. In series I, the preparations were administered 24 h prior to learning passive avoidance task; the retention of the learned response was tested 48 h afterwards. In series II, animals received the preparations 48 h after training and were tested for retention 24 h postinjection.

0007-4888/99/0008-0779$22.00

The effect of AB on gene transcription was assessed as described previously [6]. The experimental (n=4) and control rats were intraventricularly injected with AB and nonimmune γ-globulins, respectively, and decapitated 24 h postinjection. Both injection and decapitation were performed under ether narcosis. The cerebral cortex was isolated and homogenized in a medium containing 40 mM Tris-HCl (pH 7.2), 100 mM KCl, 10 mM $MgCl_2$, and 0.2 mM EDTA. After addition of 3 μCi ^{3}H-uridine, the samples were incubated for 30 min at 30°C. Transcription was stopped with a solution containing 7.5 mM carbamide, 0.5% SDS, 10 mM EDTA, and 10 mM Tris-HCl (pH 8). RNA was extracted 3 times with phenol:chloroform:isoamyl alcohol (20:20:1) and 2 times with chloroform (the samples were centrifuged at 6000 rpm for 5 min after each washing), precipitated with ethanol, incubated for 2 h at -20°C and centrifuged. The sediment was resuspended in 200 μl 2 M potassium acetate and the procedure of ethanol precipitation and incubation at -20°C was repeated. Then the RNA sediments were dissolved in 700 μl 5 mM KOH and incubated overnight at 70°C. The absorption of the RNA solution at 260 nm (A_{260}) was measured and referred to the absorption at 280 nm (A_{280}). The ratio A_{260}/A_{280} was equal to 2. The RNA samples were neutralized with glacial acetic acid and transferred to vials with 5 ml toluene scintillator. The number of scintillations per minute was counted in a Beta-1 β-counter and referred to absorption values.

To study the effect of AB on translation processes, experimental (n=4) and control rats were intraventricularly injected with AB and nonimmune γ-globulins, respectively, and decapitated 24 h postinjection. Both injection and decapitation were performed under ether narcosis. The cerebral cortex was isolated and homogenized in a medium containing 100 mM KCl, 2.5 mM $MgCl_2$, and Tris-HCl (pH 7.5). Each sample was incubated with 3 μCi ^{3}H-leucine for 30 min at 37°C [3], washed with 10% trichloroacetic acid (TCA), resuspended, and centrifuged at 6000 rpm for 5 min. The sediments were washed with 10% TCA and then incubated with 5% TCA in a water bath (90°C) for 30 min. After incubation the samples were centrifuged at 6000 rpm for 5 min and washed 2 times with 96% methanol with 5-min centrifugation at 6000 rpm after each wash. The sediments were dissolved in 700 μl 5 mM KOH and incubated at 70°C for 3-4 h. Absorption was measured at 280 nm/1.4 in a SP-46 spectrophotometer. After neutralization with an equal volume of glacial acetic acid, the assays were transferred to vials with 5 ml toluene scintillator and counted for 1 min in a Beta-1 β-counter. The data were processed using Student's t test.

RESULTS

When administered 24 h prior to passive avoidance conditioning (series I), AB to SMP-69 significantly impaired memory retention 48 h after conditioning. Experimental rats spent more time in the dark compartment and less time in the light compartment in comparison with control animals (Fig. 1, *a*). In the experimental group, the latency of entering the dark compartment tended to be shorter than in the control group.

Since memory is a multicomponent process including acquisition, consolidation, retention, and retrieval, it was necessary to determine which phase was affected by AB. For this purpose in series II, AB was administered 48 h after learning. The retention tested 24 h postinjection showed no changes in rat behavior (Fig. 1, *b*). These data indicate that retention and retrieval were not affected by the AB-induced blockade of SMP-69, since at the instant of AB administration consolidation of memory traces was completed. Therefore, behavioral changes observed in series I could be attributed to impairment of memory consolidation by AB.

The experiments with RNA and protein labeled precursors revealed a noticeable effect of AB on both transcription and translation processes in nerve cells: 24 h after AB administration the content of RNA and

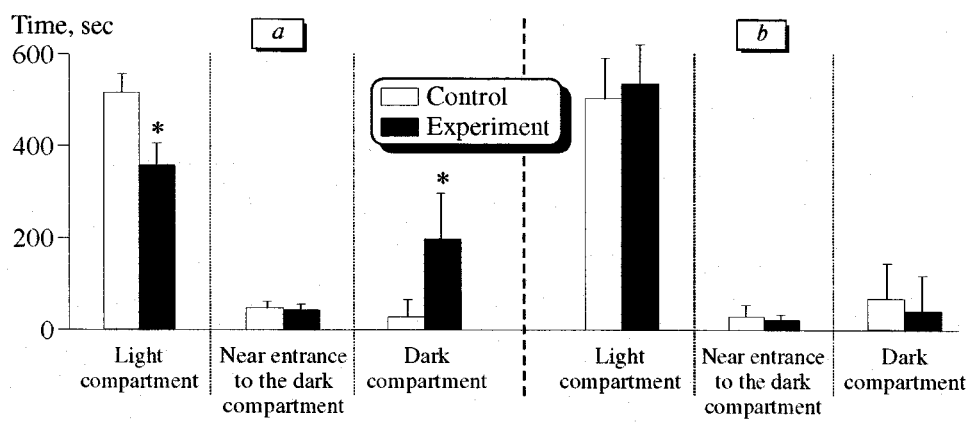

Fig. 1. Time spent in different compartments of the experimental chamber during the retention test after administration of anti-SMP-69 antibodies before (*a*) and after (*b*) learning. *p<0.05 in comparison with the control.

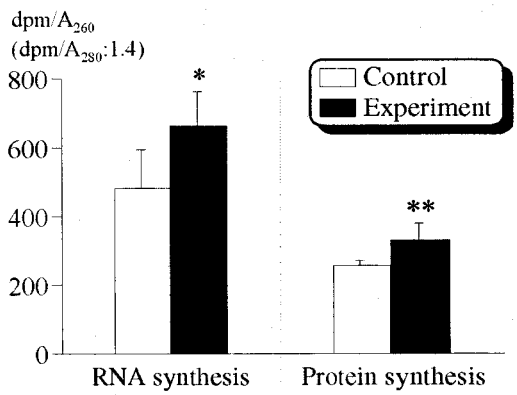

Fig. 2. Effect of antibodies to SMP-69 on RNA and protein synthesis in the cerebral cortex. *$p<0.05$; **$p<0.01$ in comparison with the control.

protein increased by 36.7% and 29.6%, respectively (Fig. 2). Thus, the impairment of memory consolidation was accompanied by genome activation. These results disagree with previous data showing genome activation in cerebral cell during memory consolidation [10,13]. It can be suggested that the blockade of SMP-69 activates genes encoding "anticonsolidation" proteins. Some brain proteins are known to perform pathological functions, in particular proteins inducing cell death in rat brain under conditions of asphyxia [11]. Under these conditions the death of experimental animals could be prevented by total inhibition of brain protein synthesis. It is possible that anticonsolidation proteins directly participate in the recording of memory traces and storing of long-term memory in cerebral cortex. When novel biologically important information enters the brain, SMP-69 probably blocks anticonsolidation genes thus allowing the signal to be recorded. If SMP-69 activity is inhibited, the activation of anticonsolidation genes leads to the synthesis of proteins preventing the recording of information. It is likely that anticonsolidation proteins are universal and regulate memory consolidation irrespective of the specificity of recorded information.

REFERENCES

1. G. G. Gasanov and A. A. Mekhtiev, *Byull. Eksp. Biol. Med.*, **112,** No. 7, 5-7 (1991).
2. Kh. Yu. Ismailova, G. G. Gasanov, T. P. Semenova, *et al., Zh. Vyssh. Nervn. Deyat.*, **39,** No. 3, 548-555 (1989)
3. M. Klemens, *Transcription and Translation. Methods* [in Russian], Moscow (1987) pp. 277-236.
4. R. I. Kruglikov, *Neurochemical Mechanisms of Learning and Memory* [in Russian], Moscow (1981).
5. A. A. Mekhtiev, G. G. Gasanov, M. M. Mekhtiev, and K. F. Zakieva, *Zh. Vyssh. Nervn. Deyat.*, **46,** No. 2, 386-388 (1996).
6. J. Manly, *Transcription and Translation. Methods* [in Russian], Moscow (1987), pp. 89-110.
7. L. A. Osterman, *Chromatography of Proteins and Nucleic Acids* [in Russian], Moscow (1985).
8. H. J. Altman, H. J. Normile, M. P. Galloway, *et al., Brain Res.*, **518,** No. 1-2, 62-66 (1990).
9. A. Barzialy, T. E. Kennedy, J. D. Sweat, and E. R. Kandel, *Neuron,* **2,** 1577-1586 (1986).
10. P. Goelet, V. F. Castelucci, S. Schachter, and E. R. Kandel, *Nature,* **322,** 419-422 (1986).
11. K. Goto, A. Ishiga, S. Kyozi, *et al., Brain Res.*, **534,** No. 1-2, 299-302 (1990).
12. J. A. Harvey, *Behav. Brain Res.*, **73,** 47-50(1995)
13. L. Kaczmarek, *J. Neurosci. Res.*, **34,** 377-381 (1993).
14. S. O. Ogren, *Acta Physiol. Scand., Suppl.*, **125,** No. 544, 2-54 (1985).
15. A. C. Santucci, P. J. Knott, and V. Haroutunian, *Eur. J. Pharmacol.*, **295,** No. 1, 7-17 (1996).