Detection of Protein with Anticonsolidation Properties in the Rat Brain

A. A. Mekhtiev

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Isolation and identification of rat brain protein regulated by serotonin-modulated protein SMP-69 is described. Intracerebral administration of anti-SMP-69 antibodies activates cerebral cortex cell genome and increases the content of electropheretic fraction 28. Intracerebral administration of isolated and purified protein to rats disturbed memory consolidation. It is concluded that nerve cells have a molecular switch regulating consolidation of memory traces.

Key Words: *cerebral cortex; genome activity; proteins; memory consolidation*

The role of different proteins in the function of nervous cells and the whole brain is usually studied by using the blockade of protein activity with monospecific antibodies [5,13,14], selective suppression of the corresponding genes with antisense nucleotide sequences, and knockout mice [9,11,12,15]. Analysis of dysfunctions resulting from the blockade or absence of certain proteins is based on the assumption that nerve cells contain the optimal quantity of different proteins necessary for their functions. Therefore, administration of these proteins can cause only minor (if any) changes in cell functions or behavioral indices. This seems to explain why protein administration is rarely used in studies of their functional activity [3]. Besides, brain proteins isolated up to now exert mainly stimulatory effects and their blockade is a more adequate approach to the analysis of their functions.

Despite numerous data on the involvement of different brain proteins in memory processes, their role in consolidation of memory traces remains unclear. Furthermore, current experimental techniques allowed to approach the understanding of the mechanisms of information storage in nerve cells, but the mechanisms regulating recording of new information in memory have not yet been explored.

A. I. Karaev Institute of Physiology, Azerbaijanian Academy of Sciences, Baku. ***Address for correspondence:*** Arifm@iphysiol.azeri.com. Mekhtiev A. A.

A new serotonin-modulated protein SMP-69 (pI 6.0, molecular weight 69 kD) was isolated from rat brain [6]. Intracerebral administration of anti-SMP-69 antibodies disturbed consolidation of memory traces during passive avoidance conditioning. We concluded that SMP-69 stimulates memory consolidation. Since memory consolidation implies the activation of cerebral cell genome and SMP-69 is thought to be a product of early genes, it was of interest to study the effect of anti-SMP-69 antibodies on genome activity and to isolate and identify proteins affected by SMP-69 blockade.

MATERIALS AND METHODS

Effect of antibodies on transcription processes was evaluated by measuring incorporation of labeled uridine. Anti-SMP antibodies (experimental rats, n=4) or nonimmune γ-globulins (control rats, n=4) were injected into the left cerebral ventricle under ether anesthesia. The rats were decapitated under ether anesthesia 24 h postinjection, the cerebral cortex was removed and homogenized in an incubation medium containing 40 mM Tris-HCl (pH 7.2), 100 mM KCl, 10 mM $MgCl_2$, and 0.2 mM EDTA. Each sample was incubated with 3 μCi ^{3}H-uridine for 30 min at 30°C. Transcription was stopped by 7.5 M urea containing 0.5 % sodium dodecyl sulfate, 10 mM EDTA, and 10 mM Tris-HCl (pH 8.0). RNA was extracted [7], absorption

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(A) was measured at 260 nm; the A_{260}/A_{280} ratio was equal 2. The RNA samples were then neutralized with glacial acetic acid, transferred to vials with 5 ml toluene scintillator, and counted on a Beta-1 counter for 1 min. The radioactivity (disintegration per minute) was referred to the absorption value.

Translation processes were studied by measuring incorporation of labeled leucine. Anti-SMP-69 antibodies (experimental rats, n=4) or nonimmune γ-globulins (control rats, n=4) were injected into the left cerebral ventricle under ether anesthesia, after 24 h the rats were decapitated under ether anesthesia, the cerebral cortex was removed and homogenized in an incubation medium: 100 mM KCl, 2.5 mM $MgCl_2$ and 2.5 mM Tris-HCl (pH 7.5). The samples were incubated with 3 μCi ^{3}H-leucine for 30 min at 37°C, then total proteins were extracted [4] and their absorption was measured at 280 nm. The protein samples were treated and counted similarly to the RNA samples, and specific radioactivity was calculated.

The same time schedule was applied for studying the effect of anti-SMP-69 antibodies on the content of some protein fractions. The experimental and control rats (6 and 5 animals, respectively) were decapitated, the cerebral cortex was removed and homogenized in cold buffer containing 0.05 M phosphate buffer (pH 7.4), 0.3 M NaCl, 0.1% Triton X-100, and 5 mM EDTA. The samples were centrifuged and dialyzed against 0.01 M phosphate buffer (pH 7.2). Proteins were fractionated by PAAG-electrophoresis in a 4-30% density gradient performed on vertical water-cooled 20×25 cm glass plates for 15 h at 0.8 mA/cm. After electrophoresis the gel was fixed with 12.5% trichloroacetic acid, stained with 0.1% CBB R-250 containing 25% ethanol and 8% glacial acetic acid, and optical density was measured on a DM-1 densitometer (Medical Equipment Factory, St. Petersburg). The content of individual protein fractions was calculated.

The proteins were isolated under the control of enzyme-linked immunosorbent assay on polystyrene plates with immunoglobulins to fraction 28. The immunoglobulins were isolated from rabbits immunized for 3 months with fraction 28 with Freund's complete adjuvant. The isolation procedure included fractional precipitation with ammonium sulfate (0-40%) followed by gel-chromatography on Sephadex G-150 columns (1.8×60 cm).

Homogeneity of isolated protein was controlled by 5% PAAG-electrophoresis in Tris-glycine buffer (pH 8.7) and its molecular weight was determined by sodium dodecyl sulfate electrophoresis using molecular weight standards.

The effect of the isolated protein on memory was studied on the model of passive avoidance conditioning. In series I, the isolated protein (15 mg/ml, 10 μl) was injected into the left cerebral ventricle of anesthetized rats (n=15) 24 h before learning. Control animals (n=12) received phosphate buffered saline (pH 7.3). During learning the animal was placed into the light compartment of the experimental chamber, entry to the dark compartment was punished with an electric shock (0.8 mA). Retention of passive avoidance reaction was tested 48 h after 5-min learning session (entry into the dark compartment was not punished). In series II (8 experimental and 7 control animals), the isolated protein was administered 48 h after learning and 24 h before the retention test.

The data were processed statistically using Student's t test.

RESULTS

The experiments with labeled RNA and protein precursors showed that anti-SMP-69 antibodies modulate transcription and translation in nervous cells: 24 hours after their intracerebral administration RNA and protein synthesis increased by 36.7% and 29.6%, respectively.

Intracerebral injection of anti-SMP-69 antibodies considerably (84%, p<0.001) increased the content of fraction 28 protein (Fig. 1, Table 1). This fraction contained protein consisting of 2 subunits with molecular

TABLE 1. Effect of Anti-SMP-69 Antibodies on RNA and Protein Synthesis and Influence of Anticonsolidation Protein on Memory Retention on Rat Model of Passive Avoidance

Index	Control	Experiment
RNA synthesis, cpm	482	663***
Protein synthesis, cpm	256	331**
Fraction 28	0.0274±0.0007	0.0503±0.0011*
The number of animals entering the dark compartment, %		
administration before learning	8.3	87.8*
administration after learning	0	25

Note. *p<0.001, **p<0.01, ***p<0.05 significant differences from the control.

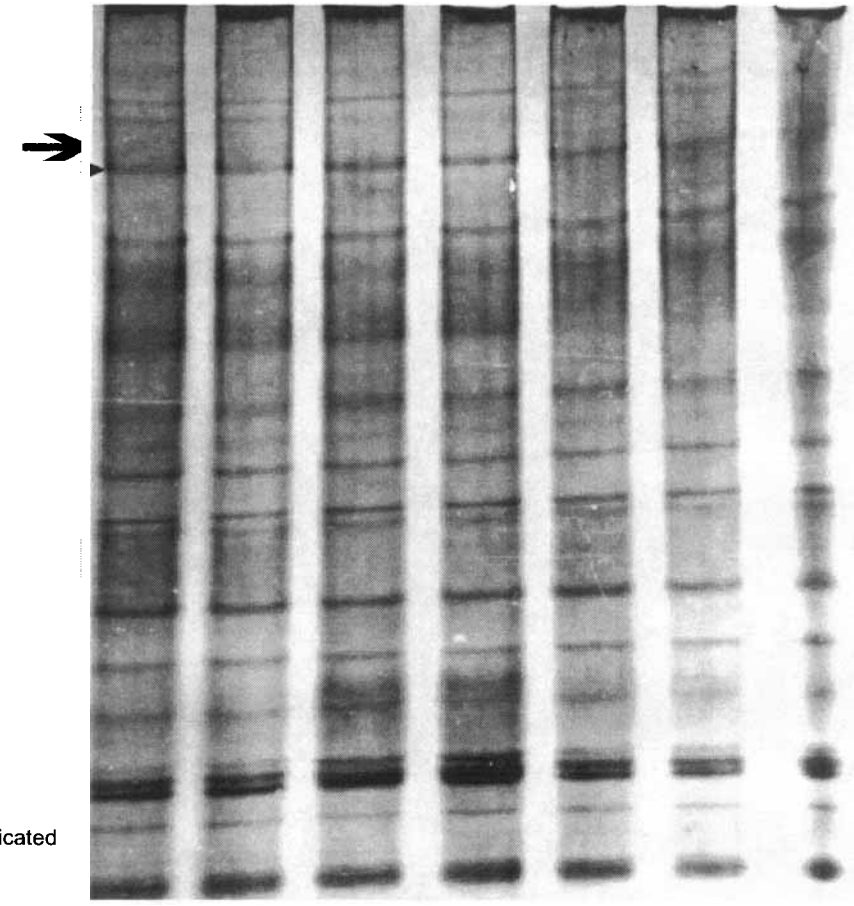

Fig. 1. Original electrophoregram. Fraction 28 is indicated by an arrow.

weights of 126 and 60 kD, respectively. Intracerebral administration of this protein 24 h before learning significantly disturbed memory processes (Table 1), while administration after learning produced no significant effects. It should be noted that the experimental rats exhibited higher exploratory activity compared to controls. It can be concluded that the isolated protein inhibits memory consolidation without affecting retention and retrieval of memory traces. Taking into account specific profile of its functional activity we called this protein anticonsolidation protein.

When analyzing these and previous data we came to a conclusion that SMP-69 and anticonsolidation protein are involved in the regulation of memory consolidation in nerve cells, but perform opposite functions: SMP-69 activates memory consolidation, while anticonsolidation protein blocks information recording. Therefore, the process of memory consolidation in the cerebral cortex is controlled by a molecular switch. The all-or-nothing principle implies the existence of a specialized substrate-matrix for the recording and storage of acquired information. Judging from the working schedule of the molecular switch mechanism, the enhancement of synaptic efficacy or the formation of new synapses could hardly be accepted as the substrate-matrix for the acquired memory. The increase in DNA synthesis reported by some investigators during learning [1,9] suggests that the acquired memory can be stored in DNA molecules. The possibility of storing acquired memory on DNA molecules during postnatal ontogeny was discussed in details by P. K. Anokhin [2]. This hypothesis is supported by the fact that the expression of unique human cortical genes increased from 8 to 38% during postnatal ontogeny, while in other cerebral structures not involved in fixation of memory trace (medulla oblongata and other tissues) the level of unique gene expression remains constant [8].

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