Monoclonal Antibodies to A3G7 Protein Associated with Nervous Tissue Growth Disturb Learning and Memory in Adult Rats

V. V. Sherstnev, M. V. Pletnikov, Z. I. Storozheva, S. G. El'nikova, T. M. Pankova, M. V. Starostina, and M. B. Shtark

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Effect of monoclonal antibodies to A3G7 protein specific for cerebellar Purkinje cells and hippocampal pyramidal neurons on learning, consolidation, and retrieval of short- and long-term habituation to acoustic startle reaction is studied in adult rats. Application of 1 mg/ml antibodies to the cerebellar vermis selectively disturbs long-term habituation, while 2 mg/ml antibodies inhibit both short- and long-term habituation. The data are analyzed with consideration of the involvement of A3G7 protein into nerve growth and neurotransmittory processes underlying learning and memory in developing and mature brain.

Key Words: *monoclonal antibodies; nerve growth factor; learning; memory; acoustic startle reaction*

This study stemmed from the concept on the involvement of molecular factors that regulate growth and development of the nervous tissue into learning and memory in mature brain [4,9,12]. Of special interest are the factors regulating the differentiation of some neurons and associated with synapto- and dendritogenesis. These processes underlie integrative functions of the nervous system both in young and adult organism [6,13].

We have previously obtained monoclonal antibodies (MAB) to neurospecific A3G7 protein localized in rat cerebellar Purkinje cells and hippocampal pyramidal neurons and involved into formation and stabilization of synaptic contacts [3]. It has been shown that anti-A3G7 MAB block induction of the long-term potentiation in hippocampal slices of adult rats and impair the active avoidance reaction in adult rats one day postinjection [1,5]. However, the role of A3G7 protein in learning and memory remained beyond the scope of these pilot studies. Taking into account the fact that A3G7 is located in cerebellar Purkinje cells, the effect of anti-A3G7 MAB was studied on the mode of habituation to acoustic startle reaction (ASR). Previous studies demonstrated that medial cerebellar structures (vermis and *nucleus fastigii*) directly participate in ASR habituation in adult rats [7,11].

The aim of the present study was to investigate the effect of anti-A3G7 MAB on acquisition, consolidation, and retrieval of short- and long-term ASR habituation in adult rats under condition of MAB application to the vermis.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 250-300 g and maintained with food and water *ad libitum* (3 rats per cage). Experiments were performed in the light period from 11:00 to 15:00 Short- and long-term habituation to ASR was used as the behavioral model. Twenty-for hours before the experiment, the animals were adapted to experimental chamber for 5 min in the absence of stimuli.

Laboratory of Functional Neurochemistry, P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow; Institute of Medical and Biological Cybernetics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

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After 24 h, the rats were placed again into the chamber and presented 6 strong acoustic stimuli with 20-sec intervals against the background of a broad-band noise and then returned to home cages. Long-term habituation was tested 24 h after learning in experimental chamber by presenting 6 acoustic stimuli with 20-sec intervals.

Startle reaction was recorded in a special chamber coupled to a recorder and computer via a tension amplifier electron system. The amplitude of ASR was measured for 100 msec after acoustic stimulation. Acoustic stimuli were generated with a power amplifier (100Y-101, Russia) through a 1GD-400P dynamic head (Russia). A 110-dB broad-band noise of 500 msec duration was presented as the stimulus, while a 72-dB noise was used as the background.

Two days before application of antibodies, an opening in rat skull over the medial cerebellar cortex was made under ether narcosis (2 mm caudally to the lambdoid suture in the projection of the frontal suture); operated rats were returned to home cage. Antibodies (5 μl) were applied onto the cerebellar cortex through the opening using a syringe with a stopper-aided needle.

For isolation of anti-A3G7 MAB, hybridoma-conditioned medium was dialyzed against saturated ammonium sulfate (1:1). After centrifugation the immunoglobulin-containing precipitate was redissolved in a minimal volume of physiological buffered saline, dialyzed against the same buffer, and applied to a chromatographic column with immobilized *St. aureus* protein A. Purified immunoglobulins were concentrated by ultrafiltration and stored at -80°C. Affinity-purified nonimmune immunoglobulins were additionally adsorbed on a column with immobilized water-soluble rat brain extract.

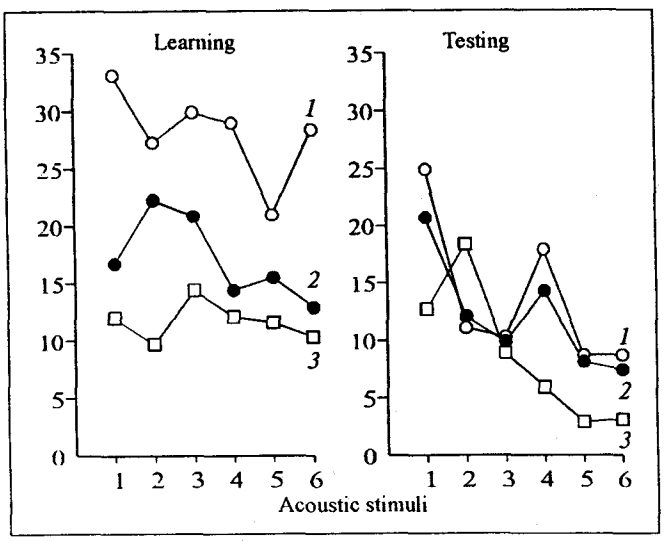

Fig. 1. Effect on anti-A3G7 monoclonal antibodies on acquisition of habituation to acoustic startle reaction (ASR) in rats. Here and in Figs. 2 and 3: ordinate: ASR amplitude, rel. units. *1*) intact animals; antibodies in concentrations of 1 (*2*) and 2 mg/ml (*3*).

Anti-A3G7 antibodies were applied 60 min before (effect on acquisition and retention of short- and long-term ASR habituation) or 2 h after learning (effect on consolidation and/or retention of long-term habituation). Antibody application 60 min before testing of long-term ASR habituation allowed us to assess the effect on its retrieval. Experimental animals were given MAB in concentrations of 1 and 2 mg/ml, while controls received the same concentrations of preimmune mouse IgG. Experimental and control groups consisted of 10 rats each. Since experimental values did not correspond to nornal distribution, the data were processed using the nonparametric sign test (Z) at $p<0.05$.

RESULTS

We have previously demonstrated that the amplitude of ASR induced by a series of strong acoustic stimuli gradually decreases from stimulus to stimulus (short-term ASR habituation phenomenon). When the same series was presented 24 h later, we observed a decrease in the amplitude of the first response in comparison with learning session and its more rapid decay during subsequent stimulation (long-term ASR habituation phenomenon) [10].

Injection of 1 mg/ml anti-A3G7 MAB 1 h before learning had no effect on short-term habituation, while a higher dose (2 mg/ml) disturbed this process. Unlike in controls, no long-term habituation was observed in experimental rats injected with 1 and 2 mg/ml antibodies.

While comparing the mean amplitude of the first 3 responses during learning and testing sessions, we found a significant decrease in this parameter after 24 h in control ($p<0.05$), but not in experimental rats (Fig. 1).

Application of anti-A3G7 MAB (both concentrations) 2 h after learning impairs the formation of long-term ASR habituation: the amplitude of the first 3 responses during learning and testing sessions differed insignificantly. Preimmune mouse IgG in concentrations of 1 and 2 mg/ml had no effect on long-term ASR habituation (Fig. 2). Since control groups did not differ from each other, we showed only one of them in Figures 1-3. The amplitude of the first 3 responses in the control groups on days 1 and 2 differed significantly ($p<0.05$).

Anti-A3G7 MAB (1 and 2 mg/ml) injected 1 h before testing had no effect on long-term ASR habituation. Both control and experimental rats demonstrated a decreased mean amplitude of the

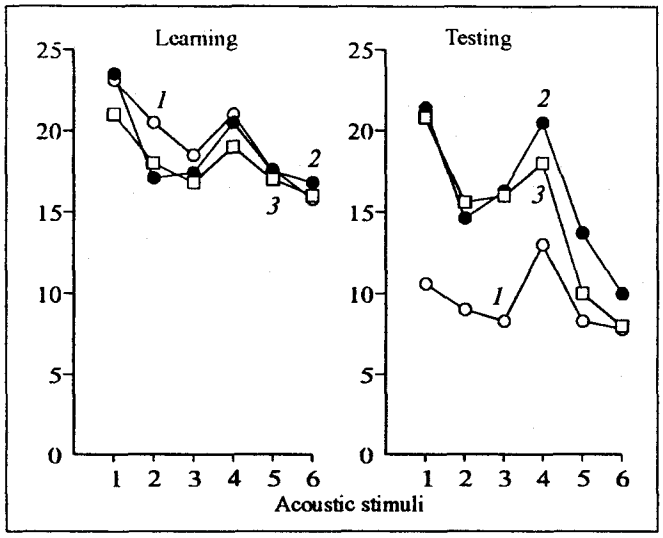

Fig. 2. Effect on anti-A3G7 monoclonal antibodies on consolidation of habituation to acoustic startle reaction in rats.

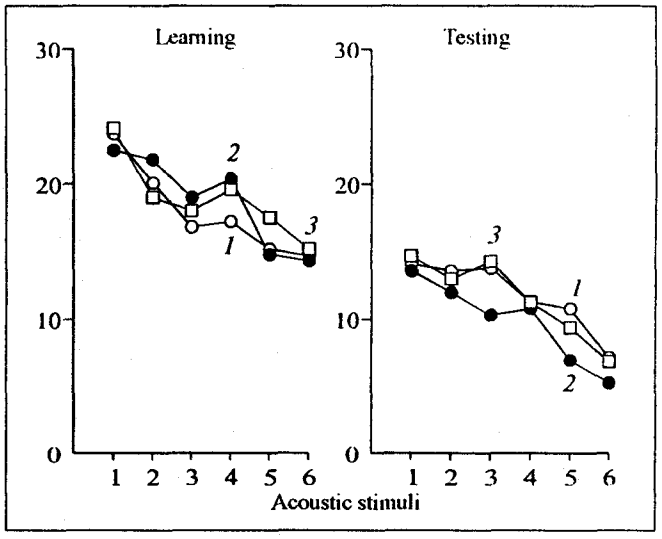

Fig. 3. Effect on anti-A3G7 monoclonal antibodies on retrieval of habituation to acoustic startle reaction in rats.

first 3 responses in comparison with learning session. The rate of ASR amplitude decay in experimental animals also remained unchanged (Fig. 3).

Thus, anti-A3G7 MAB applied to the median cerebellar cortex in a concentration of 2 mg/ml 1 h before the learning impairs both short- and long-term ASR habituation in adult rats. In a lower concentration (1 mg/ml), MAB affected only long-term ASR habituation. Injection of MAB 2 h after learning inhibited long-term habituation, while application 1 h before testing had no effect on ASR habituation.

Our findings suggest that anti-A3G7 MAB applied on the cerebellar vermis in a concentration of 1 mg/ml selectively inhibit consolidation and/or retention of long-term memory, but have no effect on learning, short-term memory, and memory retieval, while in a concentration of 2 mg/ml these MAB inhibit not only long-term, but also short-term memory and learning.

Taking into account the role of cerebellar vermis in the formation of short- and long-term ASR habituation, location of A3G7 protein on the dendrites of cerebellar Purkinje cells, and the conditions of MAB application in our experiment, it can be hypothesized that the observed behavioral changes result from the interaction between anti-A3G7 MAB and A3G7, which impairs its normal function.

It should be noted that our findings agree with the data on the role of neurospecific A3G7 protein in long-term memory in adult rats [5]. We assume that different effects of different doses of anti-A3G7 MAB are due to the involvement of A3G7 protein not only into neuron growth and differentiation [2], but also (as to other neuron growth factors, in particular, B-50 protein) into modulation of synapse functioning in mature nervous tissue [8].

Detailed discussion of the role of A3G7 protein requires further investigations. However, our findings suggest the involvement of A3G7 protein into learning and memory of adult animals and experimentally confirm the concept on universal molecular basis of the integrative function of the developing and mature brains.

REFERENCES

1. S. G. El'nikova, N. V. Egorushkina, T. M. Pankova, *et al.*, *in: Monoclonal Antibodies in Neurobiology* [in Russian], Novosibirsk (1995), pp. 93-101.
2. S. G. El'nikova, T. M. Pankova, M. V. Starostina, *et al.*, *Ibid.*, pp. 84-91.
3. S. G. El'nikova, T. M. Pankova, M. V. Starostina, and M. B. Shtark, *Ontogenez*, **23**, No. 5, 480-486 (1992).
4. S. G. El'nikova and M. V. Starostina, *Uspekhi Sovr. Biol.*, **114**, No. 3, 319-328 (1994).
5. I. F. Pavlov and M. B. Shtark, in: *Monoclonal Antibodies in Neurobiology* [in Russian], Novosibirsk (1995), pp. 102-107.
6. C. H. Baily and E. R. Kandel, *Annu. Rev. Physiol.*, **55**, 397-426 (1993).
7. M. Davis, in: *Neural Mechanisms of Startle Behavior*, New York (1984), 287-361.
8. W. H. Gispen, H. B. Nielander, P. N. E. deGraah, *et al.*, *Mol. Neurobiol.*, 5, 61-85 (1991).
9. E. R. Kandel and T. J. O'Dell, *Science*, **258**, 243-245 (1992).
10. M. V. Pletnikov, Z. I. Storozheva, and V. V. Sherstnev, *Pharmacol. Biochem. Behav.*, **54**, No. 1, 93-98 (1996).
11. Z. I. Storozheva and M. V. Pletnikov, *Neuroreport*, 5, 2065-2069 (1994).
12. S. Varon, T. Hagg, B. Fass, *et al.*, *Pharmacopsychiarty*, **22**, 120-124 (1989).
13. J. R. Wolf, R. Laskawi, W. B. Spatz, and M. Missler, *Behav. Brain Res.*, **66**, 13-20 (1995).