4. P. Moller, G. Moldenhauer, B. Mielke, *et al.*, in: *Leucocyte Typing. IV: White Cell Differentiation Antigens*, Oxford (1989), pp. 116-118.
5. A. Pezzutto, F. Behm, R. E. Callard, *et al.*, *Ibid*, pp. 165-174.
6. R. Schwartz-Albiez, B. Dorken, W. Hoffman, and G. Moldenhauer, *J. Immunol.*, **140**, № 3, 905-914 (1988).
7. Schwinzer, J. Staunton, and B. Seed, in: *Leucocyte Typing. IV: White Cell Differentiation Antigens*, Oxford (1989), pp. 628-634.

Effects of Monoclonal Antibodies on Evoked Potentials and on the Development of Long-Term Posttetanic Potentiation of the Hippocampus

T. M. Pankova, M. V. Starostina, P. N. Lyuboslavskaya, V. I. Kharchenko, and M. B. Shtark

UDC 612.821.2.06:612.124:347.96].08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 7, pp. 66-68, July, 1993
Original article submitted October 18, 1989.

Key Words: *hippocampal long-term (posttetanic) potentiation; monoclonal antibodies*

Studies of "simple" neuronal systems have shown that monoclonal antibodies (mAb) can be used for analyzing elementary physiological functions. In studies where mAb were applied to examine the involvement of brain-specific proteins in the establishment of plasticity in the central nervous system, long-term potentiation (LTP) of the hippocampus after tetanization was used as a model for this purpose. Hippocampal LTP is thought to share common molecular mechanisms with long-term memory [11], and some information has also been reported on the role played by brain-specific proteins in the origin of the LTP phenomenon [7,8].

In this study, we tested mAb that had been screened by immunohistochemical assays for their ability to serve as markers of particular nerve cell structures with a view to detecting in them not only marker properties but also inhibitory properties at the level of electrical characteristics of the hippocampus.

Department of Medical and Biological Cybernetics, Institute of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

MATERIALS AND METHODS

To obtain mAb, BALB/c mice were immunized with the synaptosomal fraction of rat cerebral cortex isolated as described previously [2], introducing 150 μg of this antigen (in terms of total protein) per injection. For hybridization, the method described by Koller and Milstein [5] was employed, and the cells were cloned by the limiting dilution technique. The hybridomas were screened by dot enzyme immunoassay [3]. The antigens identified by mAb were detected in cryostated and paraffinized sections of rat brain, using second horseradish peroxidase-conjugated antibodies to murine Ig. As the substrate for the peroxidase reaction, 3,3'-diaminobenzidine was used, and the intensity of staining was enhanced by adding sodium chloride [4].

For electrophysiological tests, Wistar rats were fixed in stereotaxic apparatus under Nembutal anesthesia (50 mg/kg), and monopolar recording electrodes and exciting bipolar electrodes 150 μ in diameter made of steel wire in Teflon insulation, were implanted into their brain ventricles together

0007-4888/93/0007-0846$12.50

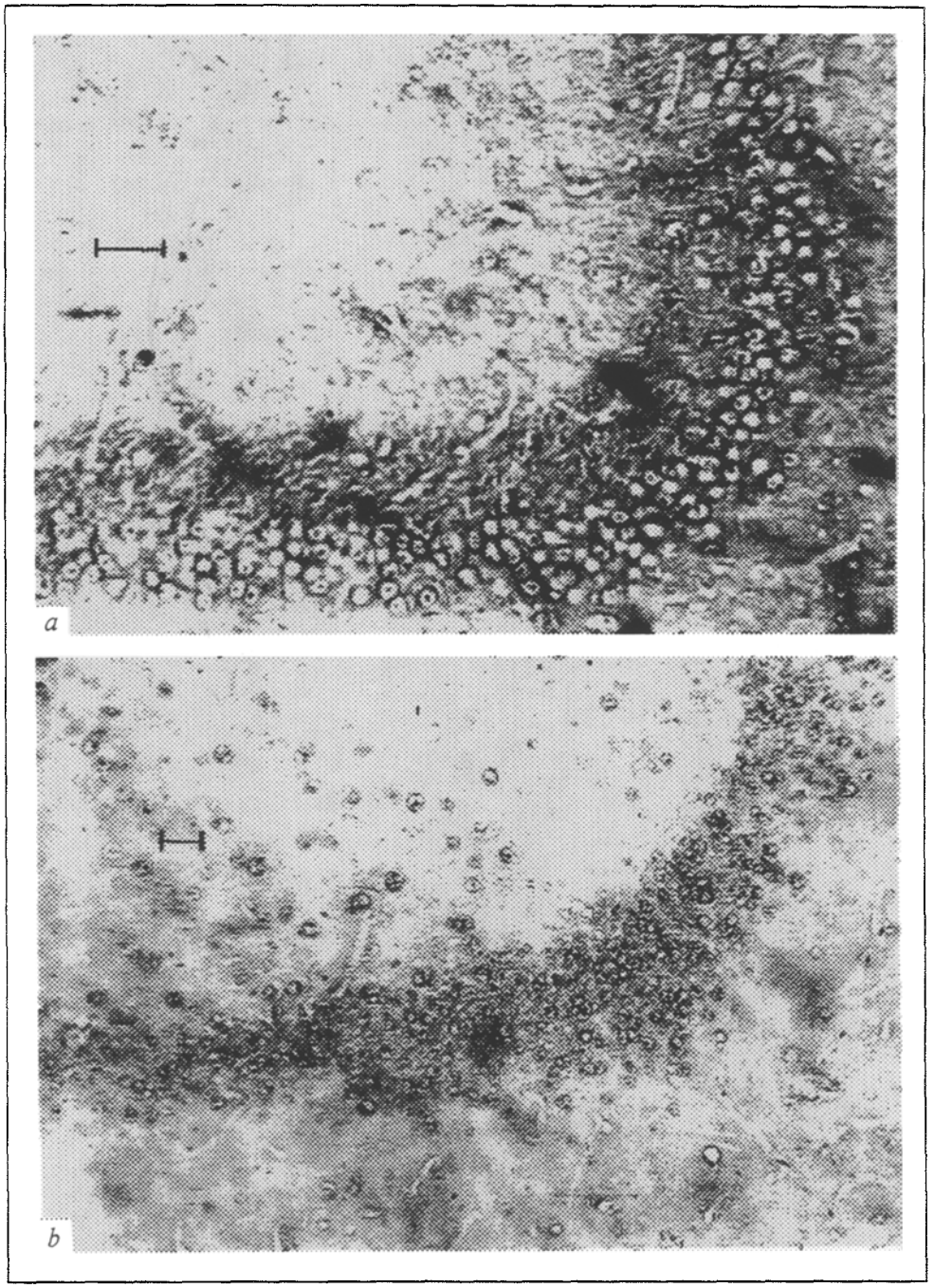

Fig. 1. Immunocytochemical localization of antigens by mAb in transverse slices of adult rat brain. *a*) staining of the neuropile of the hippocampal molecular layer by mAb 5F5–B6 (— = 100 μm); *b*) staining of hippocampal pyramidal neuronal bodies by mAb 2C9–F10.

with cannulas for intraventricular injection of the test solutions, using stereotaxic coordinates of the rat brain described by Skinner [9]. The recording electrode was placed in the area of the dentate fascia and the exciting electrodes in the zone of the perforant pathway. Their placement was checked and if necessary adjusted by noting the shape of evoked potentials (EP) [6]. The tests were started one week after the operation, using the mAb produced by clone 5F5-B6. Physiological saline and a hybridoma-conditioned medium served as control solutions. The mAb did not react positively with the antigens of rat nerve tissue. The test solutions (10 μl of each) were injected into the brain ventricles with a microsyringe at the rate of 1 μl/min.

For evaluation of the effects produced by the intraventricularly injected solutions the first derivative of the descending EP phase and the population spike amplitude were utilized.

RESULTS

Of the 246 clones tested, 35 clones that gave a positive reaction in the enzyme immunoassay with the synaptosomal fraction were selected. Four of the 35 clones were subjected to a detailed immunocytochemical study using culture fluid and also ascitic fluids taken from rats injected intraperitoneally with hybridoma cells. Each of these four clones reacted specifically with rat brain slices. One of the clones (2C9-F10) stained intensely the bodies (membranes) of hippocampal pyramidal and granular cells and cortical pyramidal neurons (Fig. 1, *b*). mAb from the other three clones stained to varying degrees the cortical, hippocampal, and cerebellar neuropils.

mAb 5F5-B6, which reacted poorly with antigens of the cerebellar molecular layer and with the cortical neuropil while intensely staining the hippocampal neuropil (Fig. 1, *a*), including its mossy fibers, was selected for the subsequent electrophysiological tests.

The control solutions injected into the brain ventricles did not alter the EP recorded from the dentate fascia, nor did they affect the development of LTP over 3 h. The effects from the intraventricularly injected mAb became evident at 30 min postinjection and reached their maximum at 120 min. This mAb decreased the first derivative (by 75.4±14.2%) and the population spike amplitude (by 70±16.4%) (Fig. 2). Since in another study the effects of intraventricularly injected polyspecific antisera were also found to attain their peak values at about 120 min postinjection [1], the time course of variation in the parameters of the excitatory potential presumably reflected the elevated antibody concentration in the hippocampal area where the recording and exciting electrodes were located.

Given that, as indicated above, mAb 5F5-B6 reacted well with mossy fibers and only slightly with neuronal bodies, this mAb presumably suppressed the activity of synapses stimulating neurons of the dentate fascia.

Tetanization of the perforant pathway carried out 120 min after mAb injection did not lead, unlike in the control tests, to an increased population spike amplitude: 90 min after the tetanization, the first derivative amounted to 71.2±11.9% and the population spike amplitude to 66±18% of their control value (Fig. 2).

It should be noted that the LTP-blocking antibodies obtained by Stanton *et al.* [10], in contrast to those used in this study, did not affect EP but did detect antigen(s) on neuronal bodies.

While the present results do not permit any conclusions to be reached about the mechanisms by which mAb act at the membrane or cellular levels, they have led us to formulate several hypotheses regarding the mechanisms of their blocking action on LTP. It is hoped that further experiments utilizing more sensitive procedures, such as extracellular and intracellular recording of the electrical activity of nerve cells in hippocampal slices, will enable us to decide which of those hypotheses is correct.

In future, work in this area should result in the production of antibodies that will block selectively the antigens involved in the pre and postsynaptic mechanisms of LTP.

REFERENCES

1. M. B. Shtark, E. V. Shevchuk, and V. V. Vyazovoi, *Byull. Eksp. Biol. Med.*, **84**, № 8, 158-160 (1977).
2. L. A. Autilio, S. H. Appel, P. Pettis, and P. L. Cambetti, *Biochemistry*, **7**, № 7, 2615-2622 (1968).
3. R. Hawkes, E. Niday, and J. Cordon, *Analyt. Biochem.*, **118**, № 1, 142-197 (1982).
4. S.-M. Hsu and E. Soban, *J. Histochem. Cytochem.*, **30**, № 6, 1072-1082 (1982).
5. G. KФller and C. Milstein, *Nature*, **265**, 495-497 (1975).
6. M. Krug, P. MБller, M. Wagner, *et al.*, *Brain Res.*, **360**, 264-272 (1985).
7. D. Lewis and T.J. Teyler, *Brain Res.*, **383**, 159-164 (1986).
8. N.Popov, S. Schulzeck, T.N. Pankova, *et al.*, *Biomed. Biochim. Acta*, **47**, № 2, 189-195 (1988).
9. J.E. Skinner, *Neuroscience. A Laboratory Manual*, Philadelphia (1971).
10. P.K. Stanton, J.M. Sarvey, and J.R. Moskal, *Proc. Nat. Acad. Sci. USA*, **84**, 1684-1688 (1987).
11. T.J. Teyler and P. Discenna, *Ann. Rev. Neurosci.*, **10**, № 1, 131-161 (1987).