In Vitro Effects of Bipathic Treatment with Antibodies in Ultralow Doses during Long-Term Post-tetanic Potentiation

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We studied the effects of individual or combination treatment with monoclonal antibodies 5F5-B6 in ultralow doses specifically marking mossy fibers in rat hippocampus and antibodies to S100 protein during long-term post-tetanic potentiation in hippocampal slices. The possible mechanisms of changes produced by therapeutic administration of antibodies in ultralow doses were revealed.

Key Words: *antibodies; ultralow doses; long-term post-tetanic potentiation*

Long-term post-tetanic potentiation (LTPTP) of synaptic transmission is a form of synaptic plasticity. It was hypothesized that LTPTP and long-term post-tetanic depression (LTPTD) play a role in learning and memory [3,4,13,15].

LTPTP is a convenient model for *in vitro* studying the effects of substances on various stages of intercellular communication. This model may be used to evaluate the influence of compounds in ultralow doses on neuronal systems.

The method of LTPTP with modifications [4,10, 12,16] is used to study cellular and subcellular mechanisms of synaptic transmission. This general process underlies functional activity of the brain in mammals.

The events that proceed in various stages of LTPTP include triggering of intracellular signal transduction, initiation of enzymes, activation of retrograde messengers (*e.g.*, arachidonic acid, prostaglandins, and NO), modulation of gene expression, and changes in the synthesis of proteins and neuropeptides.

Published data show that monoclonal antibodies (MAB) *in vitro* block LTPTP via the interaction with membrane proteins in the dentate gyrus and CA1 region [14]. Similar results were obtained in experiments with polyclonal antibodies to S100 protein [9] and MAT 5F5-B6 specifically marking the zone of mossy fibers in rat hippocampus [2].

Various biologically active substances in ultralow doses (ULD) obtained by the method of homeopathic potentiation modulate the effects of compounds in high doses during combination treatment [7]. This phenomenon received the name bipathy. These data formed a basis for the synthesis of potentiated ethanol (Anti-E, "Materia Medica Holding" Research-and-Production Company), which is used for treatment of patients with the alcohol withdrawal syndrome [1]. Experimental and clinical observations indicate that antibodies to S100 protein in ULD (PAB-S100) may relieve the withdrawal syndrome in patients with alcohol dependence [5].

In vitro effects of individual and bipathic treatment with potentiated antibodies 5F5-B6 and polyclonal antibodies to S100 protein were studied on the model of LTPTP to evaluate the possible neurophysiological mechanism of changes produced by potentiated antibodies (PAB) *in vivo*.

MATERIALS AND METHODS

Experiments were performed with hippocampal slices from 60 Wistar rats weighing 180-250 g. Transverse hippocampal slices (400 μ) were placed in a thermostatic chamber with flow Yamamoto medium [17] aerated by carbogen (95% O_2 and 5% CO_2). Evoked potentials were recorded after 40-60-min incubation. A stimulating electrolytically sharpened bipolar wolfram electrode was introduced into the zone of mossy fibers. A reference glass electrode (tip thickness 3-5 μ, resistance 1-2 mΩ) was filled with 2.5 M NaCl and placed in CA3 region (initial segments of apical dendrites). Testing was performed with single rectangular impulses (duration 200 μsec) delivered at intervals of no less than 5 min. The amplitude of testing stimuli varied from 10 to 30 V. Evoked potentials

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were recorded using a 12-digit analog-to-digit converter (Digidata and Axon Instruments Inc.) and processed on a computer (pClamp-6 software, Axon Instruments Inc.).

The amplitude of LTPTP-inducing stimuli was selected so that they produced a half-maximal response. Tetanization was induced with 3 consecutive trains of stimuli (frequency 200 Hz, train duration 1 sec) delivered at 2-sec intervals. The procedure of tetanization was repeated after 10 min. Evoked potentials were recorded no less than 40 min after the start of tetanization to evaluate. This technique allowed us to evaluate the induction or absence of LTPTP. A 1.5-2.0-fold increase in the amplitude of the excitatory postsynaptic potential (EPSP) persisting for no less than 20 min after the second tetanization served as a criterion for the induction of LTPTP.

Tetanization was induced in 1-2 slices from each series. Further experiments with the series were performed when we observed the induction of LTPTP. Antibodies or control solutions were added in the incubation medium. Slices were kept in the medium over a fixed time. To evaluate the effect of antibodies, slices were initially incubated for 20 min [9]. Then the time of incubation for antibodies in various dilutions was selected experimentally [6].

After experiments with antibodies in various dilutions the chamber was repeatedly washed with distilled water and ethanol and dried with compressed air.

We used monoclonal antibodies 5F5-B6 specifically marking the zone of mossy fibers in rat hippocampus (Institute of Molecular Biology and Biophysics) and monospecific rabbit antiserum to neurospecific S100 protein (Institute of Cytology and Genetics). Antibodies in ULD were obtained by the method of homeopathic potentiation. The initial solution of monoclonal antibodies or antiserum was repeatedly and consecutively diluted and shaken to obtain C6 and C200 (equivalent concentrations 10^{-12} and 10^{-400} wt %, respectively).

The solution of antibodies in ULD (100 μl) was added in a 10-ml experimental chamber. Nonimmune mouse IgG and ethanol (40 and 70%) served as the control.

RESULTS

Previous studies showed that incubation of slices in the medium containing MAB 5F5-B6 in a concentration of 8.5 mg/ml for 40 min completely blocks LTPTP induction [2]. These antibodies and antiserum to S100 protein may block the induction of LTPTP

TABLE 1. *In Vitro* Effects of Individual and Bipathic Treatment with MAB 5F5-B6 and Antiserum to S100 Protein on the Induction of LTPTP in Rat Hippocampal Slices (*M*±*m*, %)

Scheme		EPSP	
		before incubation	after incubation
Control	incubation with nonimmune rabbit antiserum (1:50) or 40% ethanol, 20 min	100.0±10.0	300.0±250.0*
Incubation with MAB 5F5-B6			
	8.5 mg/ml (40 min)	100.0±10.0	100.0±10.0
	dilution C6 (20 min)	100.0±10.0	175.0±20.0***
	dilution C200 (20 min)	100.0±10.0	150.0±10.0***
	dilution C6 (60 min)	100.0±10.0	120.0±10.0
	dilution C200 (60 min)	100.0±10.0	200.0±20.0***
	8.5 mg/ml and MAB 5F5-B6 in dilution C6 (20 min)	100.0±10.0	110.0±10.0
	dilution C6 (10 min); MAB 5F5-B6, 8.5 mg/ml (20 min)	100.0±10.0	110.0±10.0
	dilution C6 (20 min); MAB 5F5-B6, 8.5 mg/ml (20 min)	100.0±10.0	175.0±20.0***
	dilution C200 (20 min); MAB 5F5-B6, 8.5 mg/ml (60 min)	100.0±10.0	250.0±20.0**
Incubation with antiserum to S100			
	final dilution 1:50 (20 min)	100.0±10.0	100.0±20.0
	dilution C6 (20 min)	100.0±10.0	200.0±10.0**
	final dilution 1:50 and anti-S100 in dilution C6 (20 min)	100.0±10.0	115.0±10.0
	dilution C6 (10 min); anti-S100 in final dilution 1:50 (20 min)	100.0±10.0	130.0±15.0
	dilution C6 (20 min); anti-S100 in final dilution 1:50 (20 min)	100.0±10.0	200.0±15.0**

Note. EPSP: excitatory postsynaptic potential. *p<0.001, **p<0.01, and ***p<0.05 compared to EPSP before incubation.

after 20-min exposition, which indicates that their target proteins are involved in the early stage of LTPTP. These data are consistent with the hypothesis that endogenous S100b is involved in the mechanisms of signal transduction and stimulates adenylate cyclase during LTPTP induction [11].

Prolonged exposure of hippocampal slices to ULD of MAB 5F5-B6 in a dilution of C6 (but not C200, Table 1) was followed by changes similar to those observed after treatment with native antiserum. These results are consistent with published data that antibodies in physiological concentrations and ULD produce the same effect. Antibodies to S100 protein in high concentrations and ULD caused qualitatively similar changes in membranes of snail giant neurons, which differed only in the degree [8].

Incubation of hippocampal slices with PAB of MAB 5F5-B6 or anti-S100 in various homeopathic dilutions for 20 min blocked the influence of antibodies in high concentrations on LTPTP induction (Table 1). However, these changes were not observed after combination treatment with antibodies or short-term incubation (10 min, Table 1).

Our results suggest that antibodies to S100 protein *in vitro* block LTPTP induction. The inhibitory effect of antibodies is inversely proportional to their dilution. In dilutions of 1:50, C6 (equivalent concentration 10^{-12} wt %), and C200 (equivalent concentration 10^{-400} wt %) these antibodies inhibited the induction of LTPTP by 100, 60-70, and 0%, respectively. Preincubation of hippocampal slices with antibodies in homeopathic dilutions abolished the inhibitory effect of these substances in a dilution of 1:50 on the induction of LTPTP. This modifying action was observed after preincubation with antibodies in high (C200) and low dilutions (C30) containing only individual molecules of the initial substance (Avogadro's law). Probably, the common modifying action of antibodies is associated with the technology for preparation of diluted solutions (homeopathic potentiation), but not with the degree of dilution. It should be emphasized that the modifying effect was not observed without preincubation. Increasing the time of incubation was followed by a greater reproducibility of the effect.

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