Effects of Potentiated Antibodies to Brain Specific Protein S100 on the Dynamics of Long-Term Potentiation in Hippocampal Slices

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Native monoclonal antibodies to neurospecific S100 protein completely prevented the development of long-term potentiation (LTP) in rat hippocampal slices. Potentiated antiserum prepared by multiple dilutions according to homeopathic procedure ($1:10^{12}$) did not affect LTP, but abolished the effect of native antiserum when applied 20 min prior to it. Neither nonimmune rabbit serum at the same dilution nor homeopathic solvent modified the development of LTP in hippocampal slices.

Key Words: *long-term potentiation; antibodies; S100 protein; high dilutions; potentiated (homeopathic) form of drug*

Potentiated forms of drugs have been widely used in homeopathy, although little is known about their specific physiological effects.

Long-term potentiation (LTP) in hippocampal slices represents a suitable model for studying molecular mechanisms of synaptic plasticity and testing the effects of biologically active substances on synaptic transmission [3,8,9]. Induction of LTP is a Ca^{2+}-dependent process [2] controlled by Ca^{2+} regulatory proteins, including Ca^{2+}-binding S100 protein. It was shown that the development of LTP in hippocampal slices is accompanied by an increase in the content of membrane-bound S100 protein [1,7], while anti-S100 antiserum blocks the induction of LTP in hippocampal slices [6].

In this study we compared the effect of antibodies to neurospecific S100 protein (anti-S100) and their potentiated (homeopathic) form on LTP in hippocampal slices and investigated the effects of their combined application.

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MATERIALS AND METHODS

Experiments were carried out on hippocampal slices prepared from mature Wistar rats weighing 180-200 g (n=30).

Transverse (400-μ) sections of the hippocampus were perfused at 35-37°C with Yamamoto's solution [10] saturated with carbogen (95% O_2+5% CO_2). Electrophysiological recordings were started after a 40-60-min preincubation. Field potentials evoked by mossy fiber stimulation through an electrolytically sharpened bipolar tungsten electrode were recorded in the stratum lucidum of the CA3 region by glass microelectrodes (3-5 μ, 2-5 MΩ) filled with 2.5 M NaCl (Fig. 1, *a*).

Evoked potentials (population excitatory postsynaptic potentials, p-EPSP) were tested every 5-7 min with rectangular pulses of 200 μsec duration and 10-30 V amplitude. The signals were fed into a computer through a 12-bit analog-to-digital converter Digidata (Axon Instruments, Inc.), recorded and analyzed using pClamp-6 (Axon Instruments, Inc.) and Microcal Origin software.

Two tetani at the stimulus strength corresponding to the half-maximal response were delivered at a 10-

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min interval to produce LTP. Each tetanus consisted of three 1-sec trains of high-frequency (200 Hz) stimuli with 2 sec intertrain intervals.

Posttetanic changes in response were recorded for no less than 40 min after the first tetanus to verify the development of LTP. Considerable (1.5 to 2 times) increase in the pEPSP amplitude lasting no less than 20 min after the second tetanus was considered as LTP.

Before analyzing the effects of anti-S100 antibodies, 1-2 slices of each series were tested for the presence of LTP to ensure the quality of preparations for further experiments. Then the slices were incubated for 20 min in a medium containing antiserum or control substances in fixed concentrations. Twenty minutes of incubation is required for the development of anti-S100 effect on LTP [6]. In experiments with combined application of substances the incubation period was chosen experimentally. After each experiment the experimental chamber was thoroughly washed with distilled water and ethanol and then dried with compressed air.

We used native monospecific rabbit antiserum to neurospecific S100 protein (Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences) and its potentiated (homeopathic) form prepared in the Materia Medica by routine homeopathic technology (multiple dilutions and shakings). Nonimmune rabbit serum and 40% and 70% ethanol (solvent for homeopathic dilutions) served as the control.

RESULTS

The development of LTP is characterized by a considerable increase in the amplitude of pEPSP evoked by single test stimuli after tetanus. Induction of LTP was completely blocked by a 20-min incubation with anti-S100 at 1:50 dilution (Fig. 2, *a*), which agrees with previous data [6]. We observed blocking effects with a higher anti-S100 dilution, which could be explained by differences in the titers of antisera developed in different laboratories.

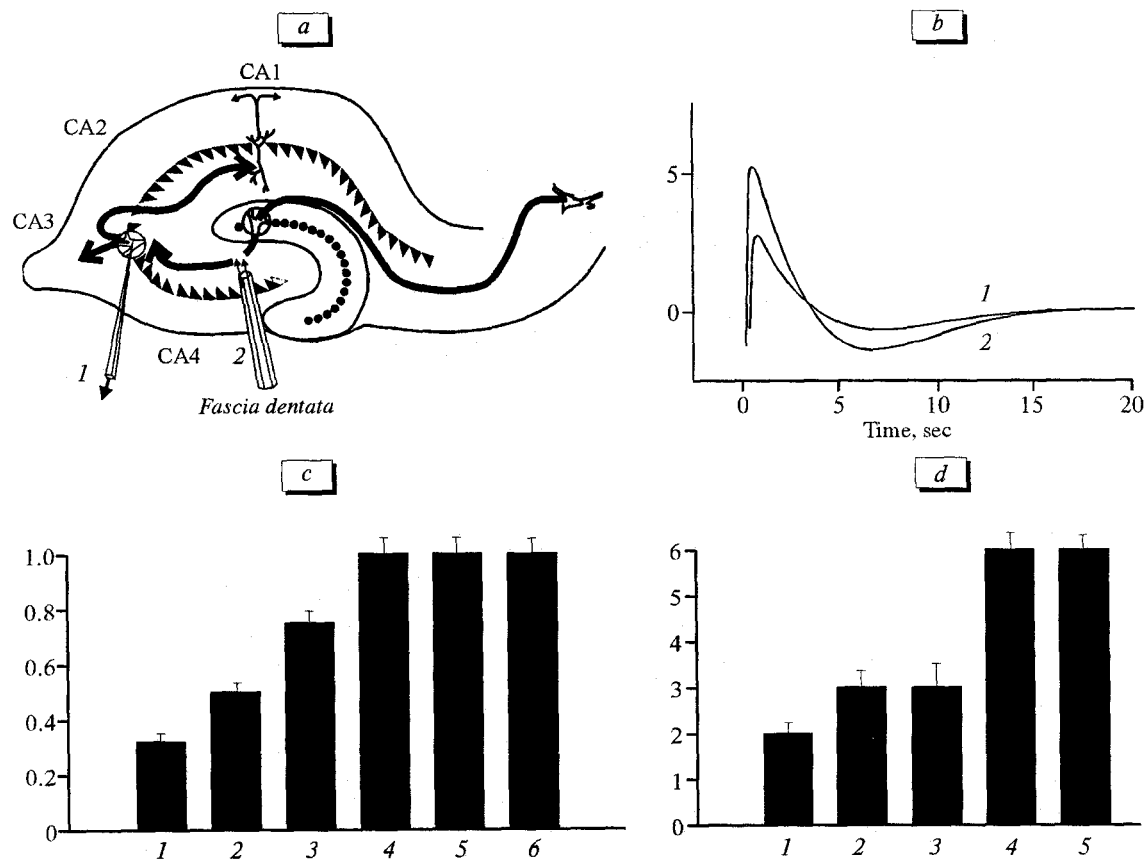

Fig. 1. Long-term potentiation (LTP) in hippocampal slices (control). *a*) location of recording (*1*) and stimulating (*2*) electrodes; *b*) field response to 20 V test stimulu in hippocampal CA3 region before (*1*) and 10 min after tetani (*2*)s. *c*) LTP development after 20-min incubation with nonimmune rabbit serum (*1*); 5 and 10 min after the first tetanus (*2, 3*) and during 20 min after the second tetanus (3 tests with 5-7 min intervals *4-6*). Serum dilution 1:50; stimulus intensity 12 V. *d*) LTP development after 20-min incubation with ethanol (40 μl of 40% ethanol added to 10 ml Yamamoto's medium); *1, 2*) two tests with a 10-min interval during incubation with ethanol; *3*) 10 min after the first tetanus; *4, 5*) 10 and 30 min after the second tetanus. Stimulus intensity 15 V. Here and in Fig. 2: ordinate is the mean amplitude of the population excitatory postsynaptic potential, mV.

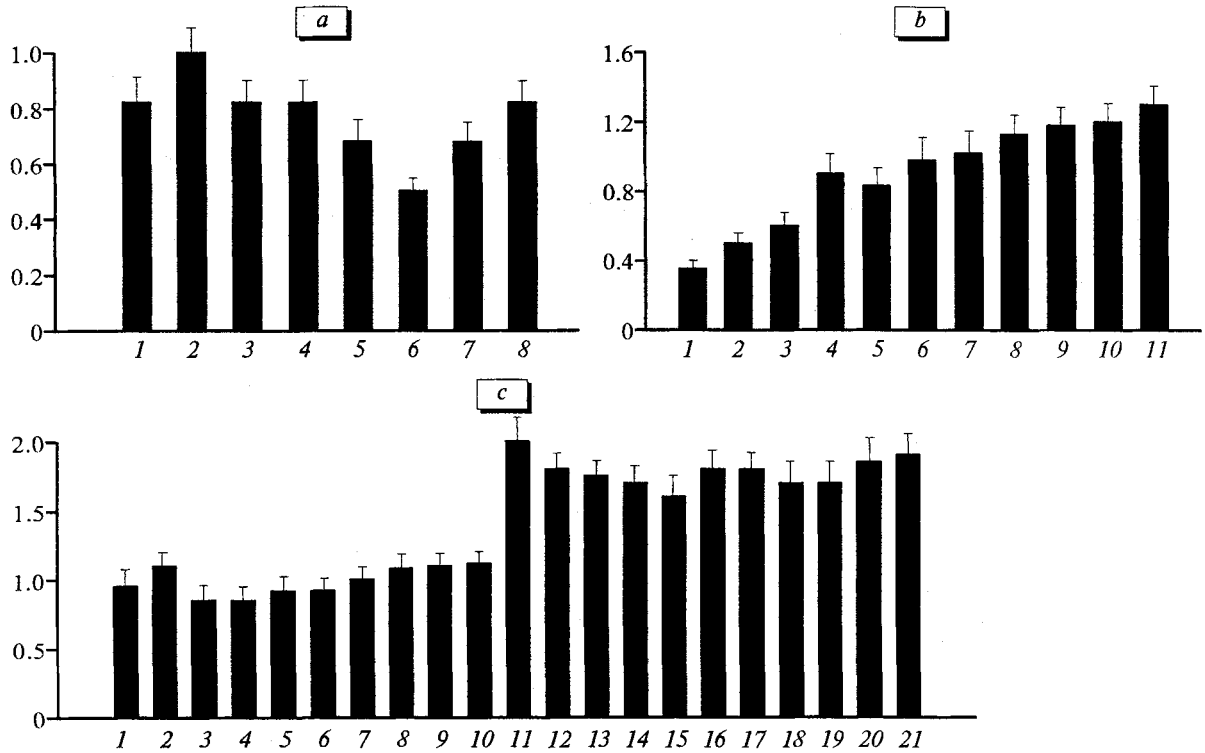

Fig. 2. "Bipathic" effects of potentiated form of anti-S100 antibodies. *a*) Absence of LTP after incubation with anti-S100 antiserum at a final dilution of 1:50; *1-3*) 20-min incubation in Yamamoto's medium with anti-S100 antiserum (test stimuli applied every 5-7 min); *4-6*) 10 min after the first tetanus (3-4-min intervals between test stimuli); *7, 8*) 10 and 25 min after the second tetanus. Test stimulus intensity 12 V. *b*) Development of LTP in the presence of potentiated anti-S100 antibodies (1:10^{12}, 40 μl); *1-3*) 20-min incubation in Yamamoto's medium with anti-S100 antibodies and (test stimuli applied every 5-7 min); *4-6*) 10 min after the first tetanus (3-4-min intervals between test stimuli); *7-11*) 30 min after the second tetanus (test stimuli applied in 5-7 min). The intensity of test stimuli 20 V. *c*) Development of LTP in the presence of anti-S100 antiserum (1:50) and potentiated antibodies (1:10^{12}, 40 μl). *1-3*) 20-min incubation in Yamamoto's medium with potentiated anti-S100 antibodies (1:10^{12}, 10-min intervals between test stimuli); *4-6*) 20 min incubation with anti-S100 (5-7-min intervals between test stimuli); *7-10*) 10 min after the fist tetanus (2-3-min intervals between test stimuli); *11-21*) 40 min after the second tetanus (responses are tested at 3-5-min intervals between stimuli); the test stimulus intensity 10 V.

Nonimmune rabbit serum at the same dilution did not affect the induction of LTP in hippocampal slices (Fig. 1, *c*).

Preincubation of slices with potentiated anti-S100 (1:10^{12}, 40 μl per 10 ml medium) or equivalent concentrations of ethanol did not prevent the development of LTP (Fig. 1, *b*, *d*).

The effects of combined application of native anti-S100 and its potentiated form were analyzed after their simultaneous and consecutive addition to the in-cubation medium. The LTP blockade was neither abolished nor reduced if anti-S100 and its potentiated form were added simultaneously or when potentiated antibodies were added 10 min before native, followed by 20-min incubation with both preparations.

At the same time, 20-min incubation of hippocampal slices with potentiated antibodies (1:10^{12}) followed by 20-min incubations with both preparations completely abolished the effect of anti-S100 antibodies and LTP was similar to that observed in control slices (Fig. 2, *c*).

The reversal of the anti-S100 effects could be caused by long (40 min) incubation with ethanol (the solvent for potentiated forms of anti-S100), which can alter membrane structures and/or modify the antigen-antibody interactions. To verify this assumption in additional experimental series the slices were preincubated with equivalent ethanol concentrations (without potentiated antibodies). It was found that under these conditions native anti-S100 preserved its ability to block LTP induction.

It has been previously demonstrated that the effects of anti-IgE antiserum on degranulation of human basophils are preserved at dilutions as high as 1:10^{35}, 1:10^{120} and 1:10^{400}, while low dilutions (1:1000) do not stimulate degranulation [5]. In our experiments, very high dilutions (1:10^{400}) had no effects on LTP induction.

However, the effect of combined application of native and potentiated antibodies (dilutions 1:10^{60} and 1:10^{400}) was observed in the models of acute and chronic alcohol intoxication and morphine abuse [4], where homeopathic forms were found to exert a protective effect. The mechanisms of these effects remain obscure and call for further investigations.

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