PHYSIOLOGY

Effect of Antibodies to Morphine on Synaptic Plasticity of the Hippocampus

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Incubation of hippocampal slices with antibodies to morphine did not change the total excitatory postsynaptic potential of mossy fibers, but markedly facilitated long-term posttetanic potentiation. Culturing of the organotypic hippocampal culture in the presence of 10 μM morphine increased the total excitatory postsynaptic potential of mossy fibers and reduced the probability of long-term posttetanic potentiation.

Key Words: *morphine; antibodies; hippocampus; long-term posttetanic potentiation*

Antibodies to nervous tissue antigens and various neurotropic compounds can affect functional activity of the central nervous system (CNS) and play a role in the pathogenesis of neurological and mental disorders [4]. Immunization during the development of drug dependence is accompanied by the formation of plasma antibodies to narcotics, *e.g.* morphine [5]. The increase in their titer underlies the development of tolerance and resistance [3]. The central effects of these antibodies remain unclear.

Previous studied showed that chronic administration of morphine increases the amplitude of evoked excitatory synaptic potentials and facilitates long-term posttetanic potentiation (LPTP) in rat hippocampal slices [8,9]. The mechanisms of these changes are poorly understood. Here we studied the role of antibodies to morphine (AM) in modification of LPTP in mossy fibers in hippocampal slices from normal rats. Organotypic culture of rat hippocampus was used to evaluate the effect of morphine under conditions not accompanied by antibody formation.

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MATERIALS AND METHODS

Hippocampal slices (400 μ) were obtained from Wistar rats weighing 150-200 g. These slices were bathed in a medium containing 124 mM NaCl, 4.9 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25.6 mM $NaHCO_3$, and 10 mM D-glucose and aerated with carbogen (95% O_2 and 5% CO_2) at 35-37°C in a thermostated flow chamber. Evoked potentials were recorded after 40-min incubation. A bipolar stimulating tungsten electrode (electrolytically sharpened) was placed in the area of mossy fibers. A recording glass electrode (tip thickness 3-5 μ, resistance 2-5 MΩ) filled with 2.5 M NaCl was introduced into CA3 region near the proximal segments of apical dendrites.

Single rectangular stimulating pulses (200 μsec, amplitude 10-30 V) were applied at intervals of no less than 5 min. Evoked potentials were recorded on a 12-bit analog-to-digital converter (Axon Instruments) and processed on a computer using pClamp-6 (Axon Instruments) and Microcal Origin softwares.

The amplitude of LPTP-inducing stimuli was selected so that they produced a half-maximum response.

Tetanization was induced with 3 consecutive trains of stimuli delivered at a frequency of 200 Hz and 2-sec intervals (train duration 1 sec). The procedure

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was repeated after 10 min. Evoked potentials were recorded no less than 60 min after tetanization. This technique allows studying of LPTP development.

Affinity purified rabbit antibodies to morphine were synthesized by M. A. Myagkova at the Institute of Biologically Active Substances. Affinity purified nonimmune rabbit immunoglobulin served as the control.

For evaluation of the influence of AM, tetanization was induced on 1-2 slices from each series. Further experiments were performed with slices demonstrating standard responses to testing stimuli and development of LPTP. The slices were incubated in the presence of AM or nonimmune immunoglobulin (final concentration 5 μg/ml) for no less than 20 min. Testing and induction of LPTP were carried out as described above.

Transverse hippocampal slices (400 μ) from 4-day-old rat pups were transferred on cover glasses coated with collagen (18 mm^2) and placed in Petri dishes to obtain the organotypic culture of the hippocampus. The culture medium contained 25% Hanks solution, 10% fetal bovine serum, 65% Dulbecco's modified Eagle medium, 0.2-0.3 U/ml insulin, 0.29 g/liter L-glutamine, and 6.5 mg/ml glucose. The slices were cultured in a CO_2 incubator at 5% CO_2, 90% humidity, and 36°C [2]. Antimitotic preparations 5-fluoro-2-deoxyuridine, cytosine-β-D-arabinofuranoside, and uridine (Sigma) in a final concentration of 10^{-6}-10^{-7} M were added to the culture medium on day 3 of culturing to suppress division of macrophages and fibroblasts that impaired organotypic characteristics of the slice.

Culturing of experimental slices was performed in the presence of 10 μM morphine. Control slices were cultured in the standard culture medium. Morphine was added to the culture medium starting from day 4 of culturing. The medium was replaced at 2-day intervals.

During electrophysiological assay the glasses with oriented explants at various stages of culturing were placed in a thermostatic flow chamber with the medium not containing morphine (similarly to slices). Stimulating and recording electrodes were introduced after 40-min preincubation. The scheme of experiments was similar to that for hippocampal slices.

RESULTS

Incubation of hippocampal slices from adult rats in a medium containing AM or nonimmune rabbit immu-

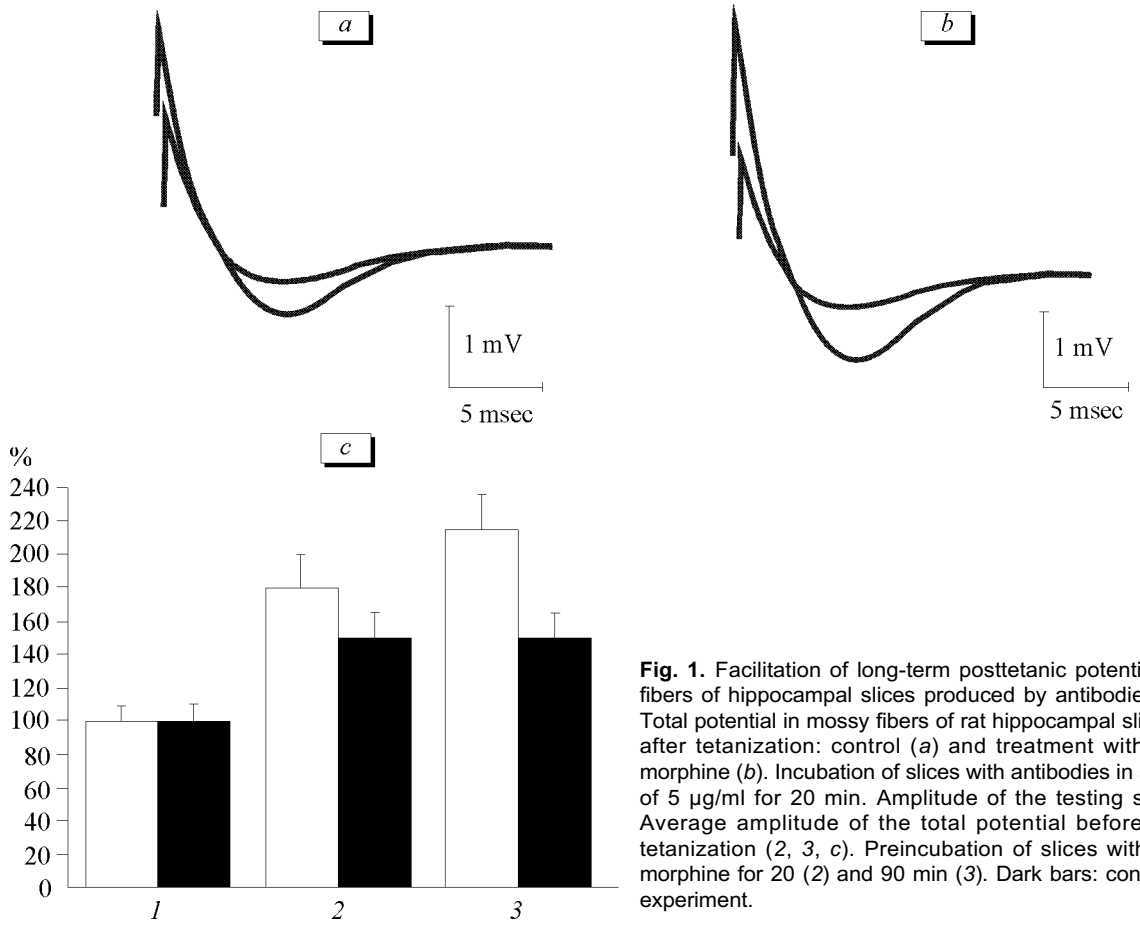

Fig. 1. Facilitation of long-term posttetanic potentiation in mossy fibers of hippocampal slices produced by antibodies to morphine. Total potential in mossy fibers of rat hippocampal slices before and after tetanization: control (*a*) and treatment with antibodies to morphine (*b*). Incubation of slices with antibodies in a concentration of 5 μg/ml for 20 min. Amplitude of the testing stimulus: 20 V. Average amplitude of the total potential before (*1*) and after tetanization (*2*, *3*, *c*). Preincubation of slices with antibodies to morphine for 20 (*2*) and 90 min (*3*). Dark bars: control. Light bars: experiment.

noglobulin (5 μg/ml) for 20 min did not change the average amplitude and latency of the excitatory postsynaptic potential (EPSP). These slices were capable of inducing LPTP. In experiments with nonimmune immunoglobulin the increase in the amplitude of total EPSP after tetanization did not differ from that observed in normal medium. However, 20-min preincubation with AM produced a more pronounced increase in the amplitude of EPSP during LPTP induction (by 10-50%, Fig. 1).

Increasing the time of incubation with AM to 90 min was followed by a more pronounced rise in the amplitude of EPSP after tetanization (30-100%, Fig. 1, *c*).

Culturing of slices in the presence of morphine had no effect on viability of the organotypic hippocampal culture. The formation of typical growth zone in control and experimental slices was observed staring from day 2 and persisted throughout culturing. This zone was formed at the peripheral area of explants and included migrating fibroblasts, astrocytes, and thin nonmyelinated axons projecting to the fimbria and region borderline to the entorhinal cortex. Binding to the substrate was not impaired.

Our previous studies showed that LPTP in mossy fibers can be induced *in vitro* starting from day 7 of culturing [1]. Morphine delayed this process: standard tetanization of mossy fibers induced LPTP no earlier than after 11-12-day culturing. LPTP was observed in 80% control explants and 40% experimental explants. The average amplitude of total EPSP in the control and experimental cultures grown in the presence of morphine increased by 3.4±1.2 and 4.9±1.4 mV, respectively.

Exogenous opiates and their agonists primarily produce an excitatory effect on neurons in the main hippocampal zones and facilitate LPTP in various systems of synaptic relationships [6-10]. The amplitude of evoked EPSP and degree of LPTP in the Schaffer's collaterals-CA1 pyramidal neurons system increased in chronically morphinized rats with naloxone-produced abstinence [8,9]. In our experiments AM facilitated LPTP in hippocampal slices from non-morphinized animals. In organotypic cultures grown in the presence of morphine the amplitude of total EPSP increased, but the ability to induce LPTP decreased.

Our experiments show that AM modify synaptic effectiveness of mossy fibers in the hippocampus. The data suggest that antibodies formed during chronic morphinization play a role in modification of synaptic plasticity.

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