Effect of Antibodies to Morphine in Ultralow Doses on Induction of Long-Term Potentiation in Hippocampal Slices from Rats with Chronic Morphine Dependence

N. A. Beregovoi, N. S. Sorokina, M. V. Starostina, M. B. Shtark, and O. I. Epstein*

Antibodies to morphine produced after its chronic administration can contribute to changes in the central nervous system during opiate abuse. Facilitation of long-term posttetanic potentiation in mossy fibers of the hippocampus in rats with chronic morphine dependence can be reproduced in hippocampal slices from normal animals treated with antibodies to morphine. Incubation of hippocampal slices with ultralow doses of antibodies to morphine had no effect on control rats, but reduced facilitation of long-term potentiation in hippocampal slices from animals with chronic morphine dependence. This confirms the possibility of using ultralow doses of antibodies to morphine for therapeutic correction of mechanisms underlying the formation of drug abuse.

Key Words: *hippocampus; long-term posttetanic potentiation; chronic morphine dependence; ultralow doses; antibodies to morphine*

Antibodies to nervous tissue antigens and various neurotropic compounds can modulate functional activity of the central nervous system (CNS) and play a role in the pathogenesis of neurological and mental disorders [2]. Immunization produced by chronic administration of drugs is accompanied by the formation of plasma antibodies to narcotic substances, *e.g.*, morphine [3]. The increase in their titer is considered as a mechanism responsible for tolerance and resistance [1]. At the same time the central effects of these antibodies remain unclear.

Formation of opiate dependence is accompanied by changes in functional characteristics of neuronal networks of the hippocampus. Chronic administration of morphine increases the amplitude of evoked excitatory synaptic potentials and facilitates long-term posttetanic potentiation (LTPTP) in various synapses of the hippocampus [5,8,9,11,12].

Previous studied showed that potentiated antibodies to S-100 protein abolish the inhibitory effect of native antibodies to this protein on LTPTP induction in the hippocampus *in vitro* [4]. This phenomenon was named "bipathic effect". Antibodies to S-100 were used for creation of an effective and safe preparation for the therapy of alcohol abuse and abstinence [7]. Autoreactivity against S-100 protein increases during alcoholism [7], therefore the effect of potentiated anti-S-100 antibodies can be explained by the bipathic phenomenon (administration of a potentiated form of preexisting in the body natural autoantibodies).

Here we studied whether antibodies to morphine can modulate LTPTP in mossy fibers of hippocampal slices from normal rats and animals with chronic morphine dependence.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats kept in cages (2 animals per cage) and feeding a standard diet. Chronic morphine dependence was modeled as described elsewhere [8]. Experimental rats received morphine with drinking water; 3% sucrose was added to mask bitter taste. Control animals drank water containing 3% sucrose. The concentration of morphine in drinking solution increased by 0.1 mg/ml every 48 h (from 0.1 to 0.4 mg/ml) and then remained unchanged

Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; *"Materia Medica Holding" Research-and-Production Company, Moscow

0007-4888/03/supp-0026$25.00

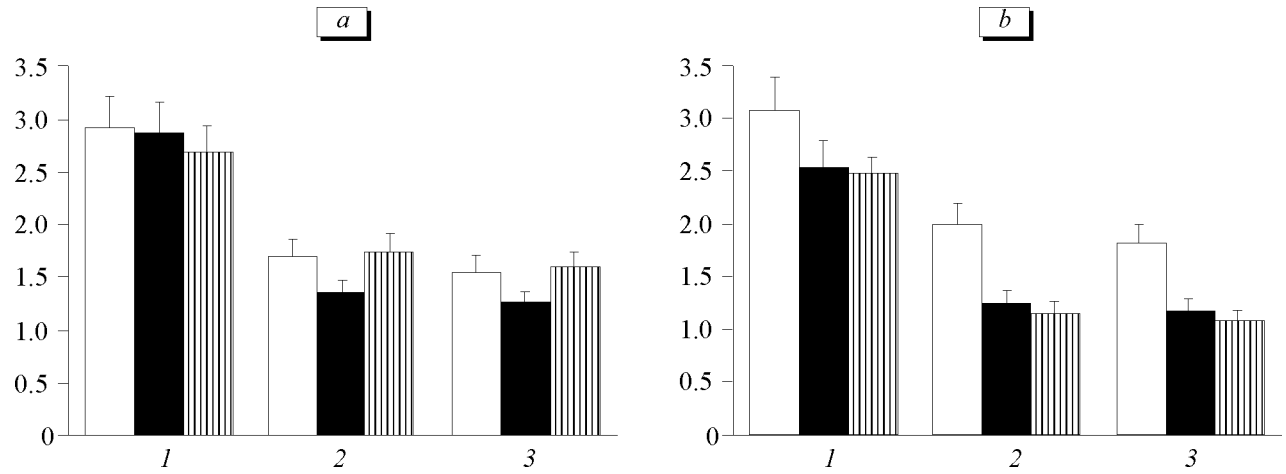

Fig. 1. Effect of potentiated antibodies to morphine on long-term posttetanic potentiation in hippocampal slices from normal rats (*a*) and animals with chronic morphinization (*b*). Light bars: control. Dark and shaded bars: incubation with antibodies for 20 and 100 min, respectively. Ordinate: changes in the amplitude of excitatory postsynaptic potential from baseline level (before tetanization). *1*) immediately after stimulation; *2*) 30 min after stimulation, *3*) 60 min after stimulation.

to the end of narcotization (0.4 mg/ml). The mean daily consumption of morphine was 40 mg/kg. Chronic morphine dependence was verified in 1-2 rats by the development of withdrawal syndrome after intraperitoneal injection of 2 mg/kg naloxone. The rats with abstinence (tremor, grooming, "wet dog shakes", head trembling, and diarrhea) were used in electrophysiological experiments. Chronic dependence usually developed over 25-30 days.

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

Potentiated antibodies to morphine (PAB-M) containing antibodies in an equivalent concentration of 10^{-12} wt % were obtained by routine homeopathic technique. Electrophysiological experiments were performed as described previously [4].

The induction of LTPTP was verified in 1-2 slices from one rat. Then other slices were used to estimate the effects of native antibodies to morphine or PAB-M. The slices were incubated with PAB-M (50 μl/10 ml) for 20-60 min. Antibodies to morphine or nonimmune immunoglobulin were added to the incubation medium to a final concentration of 5 μg/ml and incubation was carried out for 20 min or longer. Further tests and induction of LTPTP were performed as described elsewhere [4].

RESULTS

Administration of morphine with drinking water does not require daily handling and is not associated with stress (in contract to daily injections [10] or surgeries during implantation of a capsule [11]).

Antibodies to morphine appear in the blood on days 10-12 of morphinization [1]. Thus, morphinization schedule used in our experiments guarantee the presence of antibodies to morphine in experimental rats.

Incubation of hippocampal slices from normal rats in the medium containing PAB-M or nonimmune rabbit immunoglobulin for 20 min did not change the average amplitude and latency of excitatory postsynaptic potential (EPSP). Induction of LTPTP in these slices was preserved in both cases. In experiments with nonimmune immunoglobulins the increase in EPSP amplitude after tetanization did not differ from that observed in normal media. However, 20-min preincubation with PAB-M produced a much greater increase in EPSP amplitude (by 25-50%) during LTPTP induction (Table 1). Increasing the time of incubation with PAB-M to 90 min led to a more pronounced rise in EPSP amplitude after tetanization (Table 1).

This effect resembles facilitation of LTPTP in animals with chronic morphinization [8,9]. The data suggest that antibodies produced during chronic morphinization modify synaptic plasticity of the hippocampus in rats with chronic dependence on morphine.

TABLE 1. Effect of Preincubation with PAB-M on LTPTP Induction in Hippocampal Slices (*M*±*m*)

Period	Mean amplitude of EPSP, %	
	control	PAB-M
Before tetanization	100±10	100±10
After preincubation with antibodies 20 min	150±15	175±15**
90 min	150±15	200±20*

Note. *p<0.01 and **p<0.05 compared to the control.

Incubation of slices from control and morphine-treated rats with PAB-M (C6) did not change the baseline parameters of EPSP in mossy fibers. In control rats 20-min preincubation of slices with PAB-M markedly decreased the amplitude of EPSP after tetanization, which sometimes led to the absence of LTPTP. However, increasing the time of preincubation to 40-90 min abolished this effect. Therefore, incubation of hippocampal slices from control rats with PAB-M did not prevent the induction of LTPTP in mossy fibers (Fig. 1, *a*).

In hippocampal slices from rats subjected to chronic morphinization we observed facilitation of LTPTP in mossy fibers. Preincubation of slices with PAB-M for 20 min or longer significantly decreased the amplitude of EPSP after tetanization, i.e., abolished the effect of facilitation (Fig. 1, *b*). In these rats the mean amplitudes of EPSP after tetanization were similar to those in control animals. Thus, PAB-M normalized this reaction.

Our results show that PAB-M *in vitro* do not modulate the induction of LTPTP in hippocampal slices from intact rats, but abolish LTPTP facilitation in animals with chronic morphinization. Probably, these changes are associated with the above mentioned bipathic effect [4]. It should be emphasized that the effect of potentiated antibodies was observed in tissues *in vivo* exposed to antibodies to morphine generated during chronic administration.

These data suggest that antibodies to morphine generated during chronic morphinization contribute to changes in CNS during opiate abuse, in particular, they can play a role in modification of synaptic plasticity. The ability of PAB-M to abolish the specific effect of chronic morphinization on LTPTP *in vitro* indicates that they hold promise for the therapy of opiate abuse.

REFERENCES

1. L. V. Kalyuzhnyi, S. V. Litvinova, A. L. Kalyuzhnyi, *et al*., *Eksp. Klin. Farmakol.*, **61**, No. 1, 21-24 (1998).
2. G. V. Kryzhanovskii, S. V. Magaeva, and S. V. Makarov, *Neuroimmunopathology* [in Russian], Moscow (1997).
3. S. V. Tronnikov, N. B. Gamaleya, A. G. Veretinskaya, *et al*., *Byull. Eksp. Biol. Med.*, **114**, No. 2, 624-626 (1992).
4. O. I. Epshtein, N. A. Beregovoi, N. S. Sorokina, *et al*., *Ibid*., **127**, No. 7, 317-320 (1999).
5. F. Briendle, B. E. Derrick, S. B. Rodrigues, *et al*., *Brain Res. Bull*., **33**, No. 1, 17-24 (1994).
6. B. E. Derrick and J. E. Martinez, *J. Neurosci*., **14**, 4359-4367 (1994).
7. B. D. Jankovic, S. Jakulic, and J. Horvat, *Clin. Exp. Immunol*., **49**, No. 3, 598-602 (1982).
8. F. A. Mansouri, F. Motamedi, Y. Fathollahi, *et al*., *Brain Res*., **769**, No. 1, 119-124 (1997).
9. F. A. Mansouri, F. Motamedi, and Y. Fathollahi, *Ibid*., **815**, No. 3, 419-423 (1999).
10. J. L. Stringer, L. J. Greenfield, J. T. Hackett, *et al*., *Ibid*., **280**, No. 1, 127-138 (1983).
11. T. J. Wimpey, R. M. Caudle, and C. Chavkin, *Neurosci. Lett*., **110**, No. 3, 349-355 (1990).
12. W. Xie and D. W. Lewis, *J. Pharmacol. Exp. Ther*., **256**, 289-296 (1991).