Participation of L-Type Voltage-Gated Calcium Channels in Facilitation of Long-Term Potentiation during the Formation of Morphine Dependence in Rats

N. A. Beregovoy, N. S. Sorokina, and M. V. Starostina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 150, No. 8, pp. 166-169, August, 2010
Original article submitted July 30, 2009

Parameters of long-term potentiation in the system mossy fibers–CA3 pyramidal neurons in hippocampal slices in experimental animals vary during the formation of chronic opiate dependence. During the first day of morphine treatment, the value of potentiation was significantly lower than in controls. Starting from day 8 and at early stages of dependence (days 25-29), facilitation of long-term potentiation was recorded. Incubation of the slices with L-type Ca^{2+}-channel blocker nifedipine did not change the response to the test stimuli and did not affect potentiation induction in hippocampal slices from control and morphine-treated animals. Nifedipine had no effect on long-term potentiation of mossy fibers in the control and at early terms of morphine treatment, but significantly reduced its facilitation at later terms.

Key Words: *hippocampus; long-term posttetanic potentiation; morphine dependence; voltage-gated calcium channels; nifedipine*

Synaptic plasticity provides the basis for adaptation changes developing in various structures of the brain during long-term drug abuse. Published data suggest that the development of morphine dependence is related to facilitation of long-term potentiation (LTP), *i.e.* generation of postsynaptic potentials of greater amplitude after tetanization compared to intact animals [8,11]. We previously demonstrated that modification of synaptic plasticity of mossy fibers (MF) in the hippocampus under the effect of morphine had a complex pattern and preceded the development of opiate dependence in experimental animals [1]. The mechanism of modification of synaptic plasticity leading to LTP facilitation remains unknown.

When analyzing possible mechanisms of LTP facilitation in the Shaffer collaterals–CA1 pyramidal neurons system, Iranian researchers detected a considerable contribution of L-type voltage-gated Ca^{2+}-channels (L-VGC) into facilitation development in morphine-dependent animals [11,12]. LTP of hippocampal MF is mediated by other processes, primarily presynaptic processes depending on the increase in transmitter release. Various Ca^{2+}-channels are involved into transmitter release [7,10], including L-VGC, although their contribution is low [15]. At the same time, high density of L-VGC is characteristic of somatic membrane and proximal dendrites of CA3 pyramidal neurons [6], which implies their participation in modification of LTP during the development of morphine dependence.

Here we studied the role of L-VGC in LTP facilitation in the system of synaptic contacts between MF and hippocampal CA3 pyramidal neurons (MF–CA3) in animals with chronic morphine dependence and during the formation of this dependence.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 140-160 g. The animals were maintained in

Institute of Molecular Biology and Biophysics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** ber@soramn.ru. N. A. Beregovoy

0007-4888/10/1502-0203 © 2010 Springer Science+Business Media, Inc.

individual cages under standard illumination regimen (12 h day/12 h night) with free access to food. For modeling chronic opiate dependence in experimental animals, morphine was added to drinking water containing 2% sucrose. Morphine concentration was changed according to the following protocol: 0.1, 0.2, 0.3 mg/ml for 48 h each and then 0.4 mg/ml until the end of treatment [1]. Controls received 2% sucrose. The animals were used in electrophysiological experiments on days 2, 8-10, 14-15, and 21-29 of morphine treatment.

Transverse hippocampal slices (400 μ) were placed into a flow chamber with a medium of the following composition (in mM): 124 NaCl, 4.9 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 25.6 $NaHCO_3$, 10 D-glucose (pH 7.5) aerated with carbogen (95% O_2 and 5% CO_2); before the experiments, the slices were adapted to *in vitro* conditions at least 40 min at room temperature. Stimulation was performed with bipolar electrolytically sharpened tungsten electrodes positioned in the MF area. The evoked field potentials were recorded using glass microelectrodes filled with physiological saline (3-6 MΩ) placed into the area of apical dendrites of CA3 hippocampal field. For LTP development, the amplitude of the stimulus evoking half-maximum response was chosen. Tetanization was performed with three successive bursts of stimuli with a frequency of 200 Hz (1 sec burst duration and 2 sec interburst interval). Tetanization was repeated after 10 sec. The evoked field potentials were recorded for at least 60 min after the second tetanization.

For inhibition of L-VGC, the slices were incubated in the presence of 10 μM nifedipine (Sigma) for 30 min and the evoked field potentials were recorded for evaluation of its effect on the basal characteristics. The degree of LTP was evaluated by relative changes in the amplitude of evoked potentials after tetanization calculated by the formula:

$$(A_t\text{-}A_0)/A_0)\times 100\%,$$

where A_0 and A_t are the amplitudes of the response to the test stimuli before and after tetanization, respectively.

Significance of differences between the means was evaluated using one-way ANOVA and Student *t* test.

RESULTS

The method of morphine administration with drinking water allows to avoid stress to the experimental animals and effects of handling. In rats treated according to this protocol, the first signs of the syndrome in response to naloxone administration were noted on day 21, while full-scaled picture of chronic morphine dependence was observed on day 25. Long-term period of dependence formation provides the possibility of studying the dynamics of changes in synaptic plasticity during the development of opiate dependence. In preliminary experimental series, no differences in parameters of MF LTP were noted between rats receiving water or 2% sucrose solution for 30 days.

We previously demonstrated that morphine administration considerably modified synaptic plasticity of hippocampal MF and this modification had a complex pattern and significantly precedes the development of opiate dependence in experimental animals [1]. The amplitude of evoked field potentials 60 min after tetanization was significantly reduced during the first days of morphine treatment, while appreciable LTP facilitation was observed on days 8-10 and after 21 days of morphine treatment with a transient decrease on days 14-15 [1]. These terms were chosen for evaluation of the effect of L-VGC into LTP facilitation.

In hippocampal slices from control animals, incubation with nifedipine did not change the amplitude of MF potentials in response to the test stimuli. This agrees with published data on insignificant contribution of L-VGC into synaptic transmission of mossy synapses. Although this type of channels was found in giant MF buttons [15], the release of bound Ca^{2+} [14] and calcium entry through other types of channels [7,10] plays the major role in elevation of Ca^{2+} content in presynaptic terminals.

Nifedipine also had no appreciable effect on LTP induction in MF–CA3, but the degree of potentiation was lowered (Fig. 1). These findings agree with previous reports [4] that potentiation of MF was little affected by nifedipine. It is known that LTP of MF has primarily presynaptic character [7]. Potentiation degree probably decreases due to the presence of L-VGC on giant MF buds in the hippocampus, which are assumed to mediate enhanced Ca^{2+} inflow into presynaptic terminals after tetanization [15]. Incubation with nifedipine blocking these channels contributes to the decrease in potentiation degree.

At all terms of morphinization, nifedipine had no effect on responses to the testing stimuli and induction of potentiation. On day 1 of morphine treatment, the amplitude of evoked field potentials 60 min after tetanization considerably decreased in experimental animals compared to controls not treated with morphine (151.65±14.07, n=12 and 106.92±8.39, n=6, p<0.01). In this case, similarly to the control, the contribution of L-VGC into LTP changes was insignificant (~10%).

Long-term morphine treatment considerably changed parameters of LTP in MF–CA3 system in hippocampal slices. Incubation of hippocampal slices with nifedipine reduced LTP facilitation during the formation of morphine dependence, but the main evo-

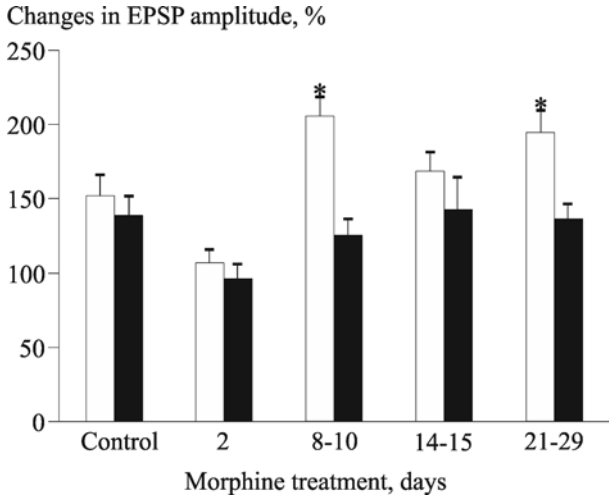

Fig. 1. Effect of nifedipine on parameters of LTP in the MF–CA3 system in hippocampal slices of the hippocampus during the formation of morphine dependence. Open bars: without nifedipine; dark bars: with nifedipine. *p<0.01 compared to the control. EPSP: evoked postsynaptic potential.

ked field potentials after tetanization remained above the corresponding values in the control group (Fig. The maximum contribution of L-VGC into LTP facilitation in MF–CA3 was recorded on days 8-10 of morphine treatment (81%); it decreased on days 14-15 (26%) and attained 58% in slices from animals with chronic dependence. Thus, L-VGC play an essential role in facilitation of LTP in the MF–CA3 system of the hippocampus during the development of physical dependence and at its early stages.

It is hardly possible that the involvement of L-VGC in LTP facilitation is determined exclusively by presynaptic channels. It cannot be excluded that the formation of morphine dependence is accompanied by changes in the contribution of pre- and postsynaptic components into the formation of LTP in the MF–CA3 system.

The involvement of L-VGC into facilitation of MF potentiation can be mediated by two not mutually excluding mechanisms: increase in their density and changes in kinetic characteristics during long-term morphine treatment. Conductivity of these channels is regulated via phosphorylation by cAMP- and Ca^{2+}-dependent protein kinases modulating the time of their open state [3,6]. Long-term action of opiates stimulates adenylate cyclase thus increasing the cAMP content in neurons and activating phosphorylation processes. This shortens the time of inactivation and increases the probability of channel open state, which, in turn, leads to manifold increase in the amplitude of Ca^{2+}-currents [3].

Activation of Ca^{2+}-currents without considerable changes in their kinetics can be related to an increase in the number of ionic channels, *e.g.* due to enhanced expression of mRNA and proteins forming these channels. Pyramid neurons of the hippocampus express two subtypes of pore-forming subunits of L-VGC: CaV1.2 (α1C) и CaV1.3 (α1D) [9], which have different biophysical properties, subcellular localization, and concentration and temporal sensitivity to dihydropyridines [2,6]. Long-term morphine treatment increases the expression of α1C, α1D, and α2/δ1 subunits of L-VGC in brain cortex, hippocampus [4,13], and mesolimbic system, including *nucleus accumbens*, *i. e.* in brain regions involved in the formation of morphine dependence. Increased expression of α2/δ1 subunits positively modifies the function of L-VGC increasing the probability of their open state [13].

The functional and quantitative changes in L-VGC observed in morphine-dependent animals in various brain areas are considered as an important neurochemical modification during the development of physical dependence; however, the role of these changes remains unclear. The results obtained in previous studies [8,11,12] and our findings suggest that this type of Ca^{2+}-channels plays an essential role in modification of synaptic plasticity of the hippocampus during the development of morphine dependence.

The study was supported by the Russian Foundation for Basic Research (grant No. 09-04-00668-a).

REFERENCES

1. N. A. Beregovoy, N. S. Sorokina, and M. V. Starostina, *Byull. Sib. Otd. Ross. Akad. Med. Nauk.*, No. 3, 153-156 (2004).
2. I. Calin-Jageman and A. Lee, *J. Neurochem*., **105**, No. 3, 573-583 (2008).
3. E. Carbone, V. Carabelli, T. Cesetti, *et al., Pflugers Arch.,* **442**, No. 6, 801-813 (2001).
4. P. E. Castillo, M. G. Weisskopf, and R. A. Nicoll, *Neuron*, **12**, No. 2, 261-269 (1994).
5. A. Díaz, J. Flórez, A. Pazos, and M. Hurlé, *Pain*, **86**, No. 3, 227-235 (2000).
6. C. J. Doering and G. W. Zamponi, *J. Bioenerg. Biomembr.,* **35**, No. 6, 491-505 (2003).
7. D. A. Henze, N. N. Urban, and G. Barrionuevo, *Neuroscience*, **98**, No. 3, 407-427 (2000).
8. F. A. Mansouri, F. Motamedi, and Y. Fathollahi, *Brain Res*., **815**, No. 2, 419-423 (1999).
9. S. Moosmang, N. Haider, N. Klugbauer, *et al., J. Neurosci.*, **25**, No. 43, 9883-9892 (2005).
10. R. A. Nicoll and D. Schmitz, *Nat. Rev. Neurosci*., **6**, No. 11, 863-876 (2005).
11. A. Pourmotabbed, F. Motamedi, Y. Fathollahi, *et al., Brain Res.,* 804, No. 1, 125-134 (1998).
12. F. Salmanzadeh, Y. Fathollahi, S. Semnanian, *et al., Ibid*., **963**, Nos. 1-2, 93-100 (2003).
13. M. Shibasaki, M. Katsura, K. Kurokawa, *et al., J. Pharmacol. Sci.,* **105**, No. 2, 177-183 (2007).
14. H. Shimizu, M. Fukaya, M. Yamasaki, *et al., Proc. Natl. Acad. Sci. USA*., **105**, No. 33, 11,998-12,003 (2008).
15. T. Tokunaga, K. Miyazaki, M. Koseki, *et al., Hippocampus,* **14**, No. 5, 570-585 (2004).