Chronic Morphine Treatment Alters Endogenous Opioid Control of Hippocampal Mossy Fiber Synaptic Transmission

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¹Vollum Institute and ²Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, Oregon 97201; and ³Actions Concertées Initiatives Jeunes Chercheurs “Plasticité Synaptique et Toxicomanie,” Centre National de la Recherche Scientifique Unité Propre de Recherche 9023, 34094 Montpellier Cedex 05, France

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Harrison, John M., Richard G. Allen, Michael J. Pellegrino, John T. Williams, and Olivier J. Manzoni. Chronic morphine treatment alters endogenous opioid control of hippocampal mossy fiber synaptic transmission. J Neurophysiol 87: 2464–2470, 2002; 10.1152/jn.00753.2001. Synaptic adaptations are thought to be an important component of the consequences of drug abuse. One such adaptation is an up-regulation of adenylyl cyclase that has been shown to increase transmitter release at several inhibitory synapses. In this study the effects of chronic morphine treatment were studied on mossy fiber synapses in the guinea pig hippocampus using extracellular field potential recordings. This opioid-sensitive synapse was chosen because of the known role of the adenylyl cyclase cascade in the regulation of glutamate release. Long-term potentiation (LTP) at the mossy fiber synapse was enhanced after chronic morphine treatment. In control animals, opioid antagonists increased LTP but had no effect in morphine-treated guinea pigs. In contrast, the long-lasting depression of transmission induced by a mGluR agonist and CA1 LTP were not altered. Chronic morphine treatment neither caused tolerance to ²μ- and ²κ-receptor–mediated inhibition at the mossy fiber synapse nor modified total hippocampal dynorphin levels. The results suggest that the phasic inhibition of glutamate transmission mediated by endogenous opioids is reduced after chronic exposure to morphine.

INTRODUCTION

Opioid agonists are known to cause presynaptic inhibition of transmitter release at many central and peripheral synapses, and recent evidence indicates that the opioid regulation of transmitter release can be fundamentally changed by chronic morphine treatment (reviewed by Williams et al. 2001). One effect of withdrawal from chronic morphine treatment that has been observed at several synapses is an increase in cAMP-dependent transmitter release (Bonci and Williams 1997; Chieng and Williams 1998; Ingram et al. 1998; Shoji et al. 1999). The mossy fiber–CA3 synapse is an opioid-sensitive site that is regulated by a cAMP-dependent mechanism (Maccarelli et al. 1998; Tong et al. 1996; Villacres et al. 1998; Weisskopf et al. 1993a,b). It is therefore of interest to examine the interaction between opioids and cAMP-dependent regulation of synaptic transmission at this synapse following chronic morphine treatment.

There has been considerable debate over the site and mechanism that underlies synaptic plasticity, particularly long-term potentiation (LTP) at the mossy fiber synapse. One unresolved issue is the role of postsynaptic calcium in the induction of LTP in CA3 pyramidal cells (Mellor and Nicoll 2001; Yeckel et al. 1999; Zalutsky and Nicoll 1990). The mechanisms that regulate glutamate release from mossy fiber terminals appear to be less conflicting. Both genetic and pharmacological manipulations have demonstrated the role of a calcium-activated form of adenylyl cyclase, and that subsequent activation of protein kinase A results in a facilitation of glutamate release (Maccarelli et al. 1998; Tzounopoulos et al. 1998; Villacres et al. 1998; Weisskopf et al. 1993a). Conversely, the inhibition of adenylyl cyclase through activation of metabotropic glutamate receptors (mGluRs) is thought to mediate a long-term depression (LTD) of glutamate release from the mossy fibers (Tzounopoulos et al. 1998; Yokoi et al. 1996).

The mossy fiber synapse is of additional interest because dynorphin, an endogenous opioid peptide, is co-released with glutamate to mediate both hetero- and homosynaptic inhibition of glutamate release (Corner-Kerr et al. 1993; Simmons et al. 1995; Weisskopf et al. 1993b). The role of endogenous dynorphin as well as exogenously applied opioids on synaptic plasticity is the subject of numerous and conflicting studies (Castillo et al. 1996; Derrick and Martinez 1994; Derrick et al. 1991; Jin and Chavkin 1999; Wagner et al. 1993; Weisskopf et al. 1993a; Williams and Johnston 1992, 1996). Issues of species variability and differences in experimental design appear to account for some of the early controversy (Salin et al. 1995); however, the role of opioids in the regulation of cellular and synaptic plasticity remains unresolved.

The purpose of the present investigation was to examine the interaction between the opioids, cAMP, and the regulation of glutamate release at the mossy fiber synapse in guinea pigs treated chronically with morphine. The choice of guinea pig was based on known presence of ²κ opioid receptors in guinea pig (Salin et al. 1995; Wagner et al. 1993; Weisskopf et al. 1993b). The results suggest that acute withdrawal from chronic morphine reduces the role of endogenous opioids in the phasic regulation of LTP.

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M E T H O D S  

Male Hartley guinea pigs (180–220 g) were anesthetized with halothane, and morphine pellets (75 mg) or sham pellets were implanted subcutaneously, one on day 1 and two on days 3 and 5. Experiments were done 7–9 days after the start of the treatment. Treatment of animals with morphine was continuous such that animals were never in a state of opioid withdrawal. This treatment protocol has been shown to produce opioid dependence and tolerance in rats and guinea pigs (Cheng and Christie 1995; Johnson and Fleming 1989; Shoji et al. 1999). Experiments done with slices from morphine-treated, sham, and untreated animals were not done blind but were interleaved.

Standard procedures were used to prepare 400-μm-thick hippocampal slices (Manzoni et al. 1995). All experiments were done after 2–6 h wash with morphine-free physiological saline. Slices were considered in acute withdrawal. Slices from naive, sham-implanted, and morphine-treated animals were termed “naive,” “sham,” and “chronic-morphine,” respectively. The superfusing solution contained (in mM) 126 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 11 glucose, and 24 NaHCO₃ and was equilibrated with 95% O₂-5% CO₂ (flow rate: 2 ml/min). Experiments were carried out at room temperature. Field potential recordings were made with electrodes filled with 3 M NaCl.

Two electrical stimuli (100 μs duration, 50-ms interval) were delivered at 0.033 Hz through bipolar tungsten electrodes placed in the dentate gyrus granule cell layer, and the recording electrode was placed in the stratum lucidum of the CA3 region. The stimulus intensity was adjusted to give a field excitatory postsynaptic potential (fEPSP) that was 50–70% of the maximal response.

Several tests confirmed that the fEPSPs were the result of stimulation of mossy fibers. First they were identified by their marked facilitation (Castillo et al. 1996; Manzoni et al. 1995). We confirmed the validity of this method to select mossy fibers [where LTP is N-methyl-D-aspartate receptor (NMDA-R) independent] (Harries and Cotman 1986) from association/commissural inputs (where LTP is NMDA-R dependent) by comparing LTP with and without dl-AP5 blockade of NMDA-R. LTP was identical in sham-operated and control guinea pigs with and without a 20-min preincubation with dl-AP5 (100 μM). Forty minutes after LTP induction, the fEPSP was 160 ± 6% (mean ± SE, n = 19) and 184 ± 16 of control (n = 7, P = 0.17, Mann-Whitney U test) without and with dl-AP5 (100 μM), respectively. Fifty minutes after tetanus, the fEPSP was 147 ± 7% (n = 19) and 172 ± 14 of control (n = 7, P = 0.13, Mann-Whitney U test) without and with dl-AP5, respectively.

An Axoclamp 2-A (Axon Instruments) was used for recordings, data were collected using ACQUIS-1 (Bio-Logic, Saint Egrève, France). fEPSPs amplitudes were measured by detecting the peak EPSP amplitude and subtracting the average value obtained during a 5-ms window immediately before the stimulus.

The levels of dynorphin(1–13) were determined using a radio immunoassay (RIA). Five hippocampal slices from a single animal were pooled in each of four control and four morphine-treated animals. The slices were extracted in 0.5 ml of 10% acetic acid containing a mammalian protease inhibitor cocktail from Sigma and 50 μg/ml bovine serum albumin. An aliquot of the extract was taken for protein determination, and both were reduced to dryness under vacuum. Acid-soluble protein was determined with a kit from Pierce (Rockford, IL). The dynorphin(1–13) RIA was from Peninsula Laboratories (RK776, Belmont, CA). The IC₅₀ was 7 pM and the sensitivity was 4 pg/tube. The antiserum shows 100% cross reactivity with Dynorphin A(1–13), porcine, Big Dynorphin(1–24), Dynorphin A, and Dynorphin A(1–12) but did not cross react with Dynorphin A(1–9), Dynorphin B or [Met]enkephalin.

All values are given as means ± SE. Statistical analyses were done with the Mann-Whitney U test using Statview (Abacus Concepts), and P < 0.05 was taken as indicating statistical significance. Drugs used were nor-Binaltorphimine 2HCl (nor-BNI), RO 20–1724, 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX) from R.B.I.; (2S,1’S, 2’S)-2’-carboxy-cyclopropyl)glycine (LCCG1), DL-α-mesoxalic acid (U69593), and naloxone from Sigma (St. Louis, MO), d-Phe-Cys-Tyr-d-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP) from Phoenix Pharmaceuticals.

R E S U L T S  

Mossy fiber long-term potentiation is enhanced during acute morphine withdrawal

Initial experiments showed that stimulation (LTP, 3 1-s trains at 100 Hz) of mossy fibers caused tetanus induced long-term potentiation that was identical in naive and sham-implanted animals. Forty minutes after LTP-induction the fEPSP was 165 ± 12% in naive (11 slices, 10 animals) and 169 ± 8% of control in sham (8 slices, 3 animals, P = 0.42, Mann-Whitney U test). Fifty minutes after tetanus, the fEPSP was 146 ± 12% in naive animals and 160 ± 9% of control in sham-implanted guinea pigs (P = 0.28, Mann-Whitney U test). Thus MF LTP was not modulated by the stress caused by pellet implantation. The data obtained from sham and naive guinea pigs were pooled in all following experiments and termed “control.”

Mossy fiber LTP was enhanced in morphine treated animals (Fig. 1, A and B). In control animals, 40 min after LTP induction the fEPSP was 168 ± 8% of baseline (n = 19, 10 animals) but was 215 ± 13% (n = 22, 9 animals) in “chronic-morphine” slices (P = 0.0022, Mann-Whitney U test, Fig. 1A). Fifty minutes after tetanus the fEPSP was 155 ± 8% of baseline (n = 19) in control animals and 202 ± 12% (n = 22) of control in “chronic morphine” slices (P = 0.0016, Mann-Whitney U test, Fig. 1A).

Morphine treatment selectively affects mossy fiber LTP

The effect of chronic morphine treatment was examined on two other synaptic processes in the hippocampus. First, morphine treatment did not change LTP measured in the CA1 region following stimulation of the Schaffer collateral pathway (Fig. 2A, control 7 animals, 5 chronic morphine animals). This suggested that chronic morphine treatment specifically altered activity-induced enhancement of synaptic plasticity at the mossy fiber synapse. Second, inhibition mediated by LCCG1, an agonist of metabotropic glutamate receptors negatively coupled to the adenylate cyclase was determined (Kobayashi et al. 1996; Tzounopoulos et al. 1998; Yokoi et al. 1996). The wash out of LCCG1 is marked by reversible and nonreversible components. The nonreversible component has been linked to long-term depression (Kobayashi et al. 1996; Tzounopoulos et al. 1998). Neither the acute nor the long-lasting inhibition caused by LCCG1 (1 μM, 3–5 min) were different (8 control and 10 chronic morphine animals, Fig. 2B). Thus chronic-morphine treatment altered mossy fiber LTP but not the long-lasting inhibition induced by LCCG1.
Acute morphine withdrawal causes an up-regulation in the cAMP-dependent cascade thought to be responsible for an increased sensitivity of the adenylate cyclase to forskolin in several brain areas (reviewed by Williams et al. 2001). The role of the cAMP cascade was examined at the MF synapse in two different experiments. Direct activation of adenylyl cyclase with forskolin (10 μM, 25 min) caused an increase in control and "chronic-morphine" slices that was not significantly different. Thirty minutes after forskolin application, the fEPSP was 353 ± 76% of baseline in control (5 animals, n = 7) and 427 ± 62% of baseline (6 animals, n = 8) in morphine-treated slices (P = 0.30, Mann-Whitney U test). The metabolism of cAMP to adenosine has been previously used to evaluate cAMP metabolism (Brundege et al. 1997; Chieng and Williams 1998; Dunwiddie and Hoffer 1980), and endogenous adenosine levels were estimated by measuring the increase of fEPSP caused by the specific A1 antagonist, DPCPX (200 nM). DPCPX caused an increase in the fEPSP in both control and chronic-morphine slices [30 min after DPCPX application the fEPSP was 340 ± 48% of baseline in control (3 animals, n = 4) and 249 ± 35% of baseline (4 animals, n = 6) in morphine-treated slices, P = 0.9, Mann-Whitney U test]. Finally, to identify the source of endogenous adenosine, the cAMP-dependent phosphodiesterase inhibitor, RO201724 (200 μM), was tested. The fEPSP was not affected by RO201724 in either group, indicating that cAMP metabolism was not a source of adenosine. In RO201724 (200 μM, 20 min) the fEPSP was 95 ± 6% of baseline in control slices (3 animals, n = 4) and 89 ± 8% (5 slices 4 animals) in morphine-treated slices.

Together these experiments suggested that neither the production nor the metabolism of cAMP were affected by morphine treatment at the mossy fiber synapse.
Phasic control of synaptic plasticity by endogenous opioids

One possible mechanism for the enhanced mossy fiber LTP observed after chronic morphine treatment was a reduction of opioid action at the presynaptic terminal. Endogenous dynorphin has been shown to cause a negative feedback through the activation of presynaptic opioid receptors to reduce glutamate release (Simmons et al. 1995; Weisskopf et al. 1993b). To test this possibility, LTP was induced in the presence of naloxone, the nonselective opioid antagonist in control and chronic-morphine slices. Naloxone (1 μM) alone had no effects on basal synaptic transmission (not shown). In the control slices, naloxone increased the amount of LTP by about 100% (4 animals, Fig. 3A). In chronic-morphine slices; however, LTP was not enhanced (4 animals, Fig. 3B). Interestingly, in slices from control animals the forskolin-induced potentiation was not changed by naloxone (3 animals, Fig. 3C), suggesting that forskolin (and the cAMP pathway) stimulates glutamate release downstream of opioid receptors.

To determine the receptor target(s) of the endogenous opioids released during tetanus, the effects of subtype-specific opioid antagonists on mossy fiber LTP were tested. Tetanus-induced LTP was increased by both μ- and κ-receptor antagonists, CTAP (1 μM), and nor-BNI (10 nM), respectively (Fig. 3, D and E). This experiment suggests that endogenous opioids act at both κ and μ receptors to depress LTP in control animals. The κ-selective antagonist diminished the early component of mossy fiber LTP but significantly enhanced the late component (Weisskopf et al. 1993b). After 40 min, the tetanus of the fEPSP was 168 ± 8% of baseline in control slices (10 animals, n = 19) and 223 ± 17% of control (3 animals, n = 5, P = 0.0027 Mann-Whitney U test) in nor-BNI treated slices. The same was observed after 50 min. The fEPSP was 155 ± 8% of baseline in control slices (n = 19) and 237 ± 21% of control (n = 5, P = 0.0075 Mann-Whitney U test) in CTAP-treated slices.

In contrast, the μ-selective antagonist CTAP augmented both early and late components. After 40 min, the fEPSP was 168 ± 8% of baseline in control slices (10 animals, n = 19) and 235 ± 35% of control (4 animals, n = 5, P = 0.011 Mann-Whitney U test) in CTAP-treated slices. Again the same was observed 50 min after the tetanus: the fEPSP was 155 ± 8% of baseline in control slices (n = 19) and 237 ± 21% of control (n = 5, P = 0.0075 Mann-Whitney U test) in CTAP-treated slices.
Lack of opioid tolerance at the mossy fiber synapse

Tolerance to endogenously released opioid is a potential explanation for the lack of action of naloxone in chronic-morphine slices (Fig. 3B). The inhibition of the fEPSP by the μ agonist, DAMGO (1 μM) and the κ agonist, U69593 (400 nM) was the same in control and chronic-morphine slices (3 animals, Fig. 4). Tolerance to the inhibitory action of μ or κ-receptor activation is unlikely to account for the lack of effect of naloxone in chronic-morphine slices.

Morphine treatment does not reduce endogenous opioid peptide levels

Chronic morphine treatment has been shown to reduce dynorphin gene expression (Rattan and Tejwani 1997; Romualdi et al. 1991). Dynorphin peptide levels assayed in the guinea pig hippocampus were not different in control and morphine withdrawn animals (control, 0.130 nM/mg acid soluble protein). Thus a simple reduction of endogenous opioid levels does not account for the reduced opioid action in slices prepared from morphine-dependent animals.

DISCUSSION

The primary observation is that mossy fiber–LTP is augmented after chronic morphine treatment. This effect was specific to mossy fiber–LTP because neither CA1-LTP nor the long-lasting depression of glutamate released induced by a mGluR agonist, at mossy fiber synapses were modified. Under the experimental conditions used in the present study, it appears that the increase in LTP may have resulted from a change in opioid-dependent facilitation. Whereas naloxone increased LTP in control, it had no effect in tissues taken from morphine-treated animals. The effect of naloxone on mossy fiber LTP has been examined by a number of different groups under a variety of conditions. The results from these studies vary from a blockade of LTP (Derrick et al. 1991; Jin and Chavkin 1999) to a facilitation of LTP (Wagner et al. 1993; Weisskopf et al. 1993b). Given that both LTP and the actions of opioids involve multiple steps and multiple sites of action, there is little wonder that results will be dependent on the experimental conditions. The results of the present work are consistent with the literature on the role of endogenous opioids on synaptic plasticity directly at the mossy fiber synapse.

It is well-known that opioids have multiple sites and mechanisms of action within various parts of the hippocampus (Castillo et al. 1996; Derrick et al. 1991; Jin and Chavkin 1999; Madamba et al. 1999; Salin et al. 1995; Simmons and Chavkin 1996; Simmons et al. 1995; Svoboda and Lupica 1998; Wagner et al. 1993). The present work has focused on presynaptic receptors located on the terminals of mossy fibers. Opioid receptors located on interneurons that are widely distributed in the hippocampus are known to have a powerful indirect, excitatory (disinhibitory) action on the pyramidal output neurons (Madison and Nicoll 1988; Nicoll et al. 1980; Zieglgansberger et al. 1979). An alternative interpretation of the present results could involve an indirect action of opioids mediated through opioid actions on interneurons (Jin and Chavkin 1999; Wimpey et al. 1989, 1990). Unfortunately there are no reports where interneurons have been examined directly after chronic opioid treatment. The direct and synaptically mediated effects of opioids on these important neurons are of obvious importance in the overall understanding of adaptive mechanisms that mediate tolerance and withdrawal from opioids.

Role of the cAMP cascade

One feature of mossy fiber–LTP is its dependence on the cAMP/protein kinase A (PKA) cascade (Huang et al. 1994; Weisskopf et al. 1993a). One surprising observation was that withdrawal from chronic morphine treatment did not affect cAMP-dependent processes at this synapse. There was no evidence for a significant up-regulation of adenylyl cyclase, since the effects of forskolin (synaptic enhancement) and LCCG1 (long-lasting depression) were similar in both groups. These observations are in agreement with biochemical experiments that did not find an augmentation of the G protein/cAMP pathways in the hippocampus in response to chronic morphine (Terwilliger et al. 1991), although the biochemical measurements may suffer from heterogeneity of cell types and adenylyl cyclase isoforms. In any case, the up-regulation of the cAMP cascade found in several opioid-sensitive synapses appears to be a common but not absolute observation (Manzoni and Williams 1999). The up-regulation of cAMP may depend on the subtype of adenylyl cyclase linked to transmitter release machinery at individual synapses.

Role of endogenous opioid peptides in the phasic control of glutamate release

A distinctive feature of the mossy fiber synapse is their ability to co-release glutamate and dynorphin (Corner-Kerr et
al. 1993; McGinty et al. 1983; McLean et al. 1987; Terrian et al. 1988; Wagner et al. 1991). High-frequency stimulation is necessary to release dynorphin, and dynorphin can act to inhibit glutamate release via presynaptic opioid receptors (Simmons et al. 1995; Weisskopf et al. 1993b). The present results extend previous reports that endogenous dynorphin regulates the threshold for LTP induction, as the \( \kappa \)-receptor antagonist, nor-BNI, decreased the stimulus threshold required to produce LTP (Weisskopf et al. 1993b). Under the conditions of the present experiments, LTP was augmented by naloxone, nor-BNI, and the \( \mu \)-selective antagonist, CTAP in slices from control animals, indicating that both \( \kappa \) and \( \mu \)-opioid receptors inhibit the field EPSP through an opioid receptor–dependent mechanism.

Adaptations of endogenous opioid peptides systems

The observation that opioid antagonists did not enhance LTP during acute morphine withdrawal could result from a number of different mechanisms. A down-regulation of presynaptic opioid receptors was ruled out since the inhibition caused by both \( \kappa \) and \( \mu \) agonists was identical in both groups. Previous studies have shown that the levels of prodynorphin mRNA (Romualdi et al. 1991) and of dynorphin (1–13) (Rattan and Tejwani 1997) were decreased in the rat hippocampus during morphine withdrawal. We found no difference between the dynorphin 1–13 levels in naive and morphine-treated guinea pigs under the conditions where clear changes in synaptic release were observed. The dynorphin RIA measured the content of peptide in a given tissue leading to the conclusion that morphine treatment did not grossly affect either the metabolism or biosynthesis of dynorphin. There are a number of conceivable ways by which chronic morphine treatment could interfere with endogenous dynorphin release without reducing total neuropeptide content. Morphine treatment could for instance reduce the dynorphin filling of dense core vesicles, the targeting of the peptide to the terminals or the release rate of dynorphin-containing vesicles in response to tetanic stimulation.

In summary, this study shows the opioid-dependent inhibitory regulation that contributes to the phasic control of synaptic plasticity at the mossy fiber synapse was reduced or eliminated by chronic morphine treatment. One potential mechanism is by a decrease in the release of a peptide co-transmitter, in this case, dynorphin. If this hypothesis holds up to rigorous testing, it offers another mechanism that may have significant impact on the regulation of synaptic function following chronic morphine treatment.

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