Chronic Morphine Treatment Selectively Augments Metabotropic Glutamate Receptor-Induced Inhibition of N-Methyl-D-Aspartate Receptor-Mediated Neurotransmission in Nucleus Accumbens

GILLES MARTIN, RISZARD PRZEWLOCKI and GEORGE R. SIGGINS
The Scripps Research Institute, Department of Neuropharmacology, La Jolla, California (G.M and G.R.S); and Neuropeptide Research Department, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland (R.P.)
Accepted for publication July 20, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
We compared the effects of different metabotropic glutamate receptor (mGluR) agonists on pharmacologically isolated N-methyl-D-aspartate-excitatory postsynaptic currents (NMDA-EPSCs) in core nucleus accumbens neurons using conventional intracellular recording in untreated and morphine-treated rats. The rats were treated by s.c. implantation of two morphine pellets and studied over a 3- to 6-day period. This model is known to exhibit opiate tolerance and dependence. We elicited NMDA-EPSCs by stimulating locally in the presence of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) and the γ-aminobutyric acid receptor antagonist bicuculline (15 μM). We found that trans-1-aminocyclopentane-1,3-decarboxylic acid, an agonist of group 1 and 2 mGluRs, decreased NMDA-EPSC areas (time-integrals) in a dose-dependent manner (1–10 μM) in slices taken from untreated rats. This inhibitory effect was significantly enhanced after chronic morphine treatment. In contrast, although the group 3 mGluR agonist L(+)2-amino-4-phosphonobutyric acid also markedly reduced NMDA-EPSC areas, there was no apparent change in this effect after chronic morphine. We found that quisqualate, the group 1 mGluR agonist, failed to elicit any effect on NMDA-EPSCs in either untreated or chronically treated rats. Paired-pulse stimulation of core nucleus accumbens NMDA-EPSCs in slices from these groups showed that chronic morphine enhanced paired-pulse facilitation, consistent with a presynaptic reduction in glutamate release. Because of the relevance to opiate tolerance and dependence of the chronic model used, the brain region (accumbens), and the receptors studied, our data provide a cellular substrate that could account for some aspects of these phenomena.

The mechanisms of tolerance and dependence to morphine have been studied extensively, in part due to the negative effects of these phenomena on long-term opiate analgesia. Despite the opioid receptor down-regulation in response to chronic morphine exposure in vitro and in vivo (Morris and Her, 1989; Ronneklev et al., 1996), there is a strong up-regulation of the cAMP system (Nestler et al., 1993). In addition to this mechanism, it also has been proposed that nitric oxide and the protein kinase C (PKC) pathways might also play a role. Thus, Mayer et al. (1995) recently showed that the development of tolerance to the analgesic effects of morphine was markedly reduced by agents blocking PKC translocation. Similarly, n(G)-nitro-l-arginine methylester, a nitric oxide synthase inhibitor, reduced the development of morphine tolerance and dependence (Majeed et al., 1994). Recently, Chen and Huang (1991) showed that the selective μ-opiate receptor agonist [d-Ala²-N-Me-Phe³,Gly-ol⁴]-enkephalin increased the amplitude of N-methyl-D-aspartate (NMDA) currents in cultured spinal cord cells, via activation of a PKC pathway that decreased Mg²⁺ blockade.

It is now thought that the NMDA glutamate receptor subtype plays a key role in opioid tolerance and dependence. Thus, the NMDA receptor antagonist MK801 reduces some aspects of these phenomena (Herman et al., 1995; Marek et al., 1991; Trujillo and Akil, 1991, 1995). In addition, recent studies (Fundytus et al., 1995; Fundytus and Coderre, 1997a, b) have pointed to the role of metabotropic glutamate receptors (mGluRs) by showing that chronic i.c.v. administration of α-methyl-4-carboxyphenylglycine, a specific antagonist of group 1 and 2 mGluRs, markedly attenuated abstinence syn-

ABBREVIATIONS: PKC, protein kinase C; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; NAcc; nucleus accumbens; mGluRs, metabotropic glutamate receptors; NMDA, N-methyl-D-aspartate; trans-ACPD, trans-1-aminocyclopentane-1,3-decarboxylic acid; EPSC, excitatory postsynaptic currents; l-AP4, L(+)2-amino-4-phosphonobutyric acid; ACSF, artificial cerebrospinal fluid.
Morphine treatment selectively alters the regulation of glutamate release via presynaptic mGlurRs. Thus, the data show that the presynaptic inhibitory effect of trans-ACPD on the NMDA current is significantly enhanced, whereas the postsynaptic inhibition mediated by t-AP4-sensitive receptors is comparable with that observed in control rats.

Materials and Methods

Animal and Slice Preparations. We used male Sprague-Dawley rats (100 and 170 g) to prepare accumbens slices from fresh brain tissue, as described previously (Yuan et al., 1992; Nie et al., 1993). We rapidly removed the brain and transferred it to a cold (4°C) and oxygenated artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl, 130; KCl, 3.5; NaH2PO4, 1.25; MgSO4·7H2O, 1.5; CaCl2, 2; NaHCO3, 24; glucose, 10. When the slices were obtained from morphine-treated but not control or placebo-treated rats, 1 μM morphine was added to the ACSF to prevent withdrawal.

Recording. We used sharp microelectrodes from borosilicate capillary glass (outer diameter, 1.2 mm; inner diameter, 0.8 mm) on a Brown-Framing puller (Sutter Instruments, Novato, CA) and filled them with 3 M KCl. Tip resistances were 60 to 100 MΩ. Our standard drug-testing protocol was as follows: after recording a stable membrane potential and NMDA receptor-evoked events for at least 15 min, we superfused the slices with the ACSF-antagonist solution described above but containing either trans-ACPD (5–10 μM; 15–25 min), quisqualate (1 μM), or t-AP4 (20 μM). Each cell served as its own control. The superfusion system allowed switching of drug-ACSF solutions without disrupting the rapid flow of the ACSF through the recording chamber (Moore et al., 1994).

Chronic Morphine Treatment. Morphine pellets (75 mg of base) and placebo pellets were provided by the National Institute on Drug Abuse. Two pellets (either morphine or placebo) were implanted s.c. in the neck in each rat under light halothane anesthesia. All electrophysiological testing was done 3 to 6 days after pellet implantation. This treatment was based on the model designed by Gold et al. (1994), who showed that it led to tolerance 12 h postimplantation, that plasma morphine levels remained constant for several days, and that withdrawal symptoms could be observed up to 13 days after implantation.

Statistical Analyses. We expressed all grouped data as mean ± S.E.M. Statistical significance between control, drug, and washout within each group of cells was analyzed with one-factor analysis of variance for repeated measures, with a post hoc analysis by Newman-Keuls or Fisher’s LSD comparison tests. We analyzed differences (expressed as percentage of control) within and between groups of cells from untreated, morphine-treated, and sham-operated rats by one-way analysis of variance between subjects. We considered p values of less than .05 statistically significant.

Results

Effect of trans-ACPD on NMDA-EPSC. As we previously reported, the group 1 and 2 metabotropic agonist trans-ACPD had no effect on membrane potential or input conductance, but markedly depressed NMDA-EPSCs in NAcc neurons (Martin et al., 1997b). Figure 1 shows the inhibition of
Fig. 1. Chronic morphine augments the depressant effect of trans-ACPD on NMDA-EPSC areas. A, Pharmacologically isolated NMDA-EPSCs from different NAcc neurons recorded at hyperpolarized and depolarized potentials in the presence of CNQX (10 μM) and bicuculline (15 μM). These recordings were obtained from slices of untreated (left panel) and morphine-treated rats (right panel). In cells of untreated rats, 5 μM trans-ACPD slightly decreased NMDA-EPSC amplitudes, whereas the depression was more robust after morphine treatment. These effects were followed by an nearly complete recovery on washout (right panel). B, In another neuron, NMDA-EPSC traces recorded before and during 10 μM trans-ACPD superfusion in NAcc neurons from untreated (left panel) and morphine-treated rats (right panel). Note the difference between the depressant effect of trans-ACPD in these two neurons. In this and subsequent figures, vertical arrows indicate time of local pathway stimulation. C, Grouped data; mean effect (±S.E.M.) in percentage of control of 5 μM trans-ACPD on NMDA-EPSC time-integrals in NAcc neurons from untreated (n = 7), morphine-treated (n = 7), and sham-operated (n = 6) rats evoked over the same range of membrane potentials from −90 to −55 mV. D, The mean effect (±S.E.M.) of 10 μM trans-ACPD on NMDA-EPSC areas from a pool of 7, 6, and 7 neurons from untreated, morphine-treated, and sham-operated rats, respectively.

NMChs were evoked by local stimulation in the presence of 10 μM CNQX and 15 μM bicuculline, recorded at holding potentials around −60 mV in slices obtained from untreated and morphine-treated rats. As previously showed, the amplitude and duration of this pharmacologically isolated EPSC component increased as the cell was depolarized, and the EPSCs were nearly completely blocked by 60 μM D-2-amino-5-phosphonovaleric-acid (Martin et al., 1997a,b). Superfusion of 5 μM trans-ACPD weakly decreased the NMDA-EPSC amplitude in untreated rats (Fig. 1A, left panel). However, the same trans-ACPD concentration applied to slices from a rat chronically treated with morphine markedly decreased NMDA-EPSC amplitudes.

Figure 1B (left panel) shows the effect of a higher trans-ACPD concentration (10 μM) on NMDA-EPSCs recorded from another cell clamped at −56 mV from an untreated rat. Under these conditions, 10 μM trans-ACPD clearly attenuated the NMDA current amplitude after chronic morphine treatment; here the inhibition elicited by trans-ACPD was consistently larger (Fig. 1B, right panel).

The grouped data also suggested that chronic morphine treatment enhances the trans-ACPD depression of NMDA-EPSC. Figures 1, C and D compare the mean decrease of the NMDA-EPSC areas elicited by trans-ACPD (5 and 10 μM) in NAcc neurons from untreated, morphine-treated, and sham-operated rats. The graphs show no clear voltage-dependent effect of 5 and 10 μM trans-ACPD on the NMDA-EPSC time-integrals in any of the experimental groups of cells. For the untreated group, 5 μM trans-ACPD depressed time-integrals by 5 ± 5 to 17 ± 13% of control (Fig. 1C). After chronic morphine treatment, this effect was greatly enhanced at all potentials; trans-ACPD decreased the responses by 20 ± 4.8 to 28 ± 3.2% of control. The possibility that surgery could have been responsible for this effect can be ruled out, because the effect of the same trans-ACPD concentration (5 μM) on a group of cells from sham-operated rats was even smaller than that on cells of the untreated group (Fig. 1, C and D). Similarly, the effect of 10 μM trans-ACPD also was augmented after morphine treatment. Thus, 10 μM trans-ACPD decreased the mean NMDA-EPSC time-integral by 31 ± 8.4% for the untreated group and by 47 ± 7.3% in the morphine group. As with 5 μM trans-ACPD, the effect of 10 μM trans-ACPD on the NMDA-EPSC time-integral cannot be attributed to the surgery, because the inhibition caused by the same trans-ACPD concentration in the sham group was even less pronounced than in cells of the untreated group.

Figure 2 shows the effect of 1, 5, and 10 μM trans-ACPD averaged over all potentials. Although 1 μM trans-ACPD did not affect mean NMDA-EPSC areas in neurons from the untreated group, it significantly decreased them by 12 ± 2.1% in neurons of morphine-treated rats (p < .001; n = 5). The difference between the two groups was statistically significant (F(1,65) = 6.5; p < .012; n = 5). At 5 μM, trans-ACPD also significantly decreased the mean NMDA-EPSC time-integrals, by 12 ± 3.9% in the untreated group (F(2,41) = 10.14, p < .003), by 25 ± 1.8% in the morphine-treated group.

Fig. 2. Dose-response relationships for trans-ACPD reduction of NMDA-EPSC time-integrals, averaged over all tested membrane potentials and plotted as percentage of inhibition of EPSC time-integral versus trans-ACPD concentration (1–10 μM), in a group of untreated (black column), morphine-treated (white column), and sham-operated rats (gray column). Numbers in columns indicate number of neurons.
(F(2,82) = 47.32; p < .0001), and by 8.2 ± 3.5% in the sham-operated group (F(2,70) = 18.14; p < .007). Statistical analyses between groups showed a significant difference between the untreated and morphine groups (F(1,68) = 6.22; p < .015) and between the sham and morphine groups (F(1,76) = 18.14; p < .0001). However, comparing the untreated and the sham groups revealed no significant difference (F(1,75) = .35; p = .55).

Figure 2 also shows that the inhibition by 10 μM trans-ACPD of NMDA-EPSC time-integrals was greater after morphine treatment. The trans-ACPD effect was significantly different under all experimental conditions, and the comparison between groups revealed that the differences in trans-ACPD effect between untreated and morphine-treated groups and between sham- and morphine-treated groups were statistically significant (F(1,63) = 10.81; p < .0017 and F(1,70) = 40.87; p < .0001, respectively).

**Effect of Quisqualate and l-AP4 on NMDA-EPSCs.** We previously reported that the group 1 metabotropic agonist quisqualate had no effect on NMDA-EPSCs in NAcc neurons (Martin et al., 1997a). In the present study we verified this lack of effect, but also now in NAcc neurons of morphine-treated rats (Fig. 3). We also re-examined the effect of l-AP4 (20 μM) on NMDA-EPSCs. We previously found that this group 3 mGluR agonist markedly depressed, probably at the postsynaptic level, NMDA-EPSCs and currents evoked by exogenous NMDA in NAcc neurons (Martin et al., 1997a). Figure 3A shows NMDA-EPSCs recorded at three membrane potentials from a cell (resting membrane potential = −84 mV) recorded in a slice from the morphine group. As with slices from untreated animals, l-AP4 markedly attenuated NMDA-EPSC time-integrals. This effect, statistically significant compared with controls (F(2,46) = 78.85; p < .001, n = 4), was followed by recovery on washout. Figure 3C shows the mean decrease of NMDA-EPSC time-integrals by l-AP4 over all potentials in four cells each from untreated and morphine-treated rats. Although the inhibitory effect of l-AP4 on NAcc neurons from morphine-treated rats was slightly greater compared with those of the untreated group, the difference was not statistically significant (F(1,41) = 16.69; p = .203). A possible explanation for this absence of significant effect between the two groups is that the inhibition elicited by 20 μM l-AP4 represents a ceiling effect that would prevent the chronic morphine treatment from further decreasing NMDA-EPSCs amplitude. However, this possibility is not likely, because the inhibition mediated by 40 μM l-AP4 in untreated rats was larger than that with 20 μM (data not shown).

**Paired-Pulse Facilitation.** Our previous studies (Martin et al., 1997a) showed that trans-ACPD probably acts presynaptically on NAcc neurons to decrease glutamate release. Therefore, our present findings suggest that chronic morphine treatment increases the presynaptic inhibitory control of mGluRs on glutamate release in NAcc neurons. To test this hypothesis further, we measured the amplitude of NMDA-EPSCs elicited by a paired-pulse paradigm, often used to discriminate pre- from postsynaptic site of drug action. Figure 4 shows that in untreated rats, an interstimulus interval of 50 ms markedly facilitated the second of two NMDA-EPSC amplitudes; in controls, the secondary NMDA-EPSC amplitudes, measured at −60 mV, were facilitated significantly to 153 ± 5.1% of control (p < .001). This effect disappeared at an

![Fig. 3.](image)

![Fig. 4.](image)
interval of 200 ms, because the NMDA-EPSC amplitude was only 95 ± 4.2% of control. Interestingly, a paired-pulse inhibition occurred in controls at an interval of 400 ms, because the amplitude of the synaptic response was attenuated by 21 ± 11%. After chronic morphine treatment, the paired-pulse facilitation observed at a 50-ms interstimulus interval was significantly enhanced: the mean amplitude of the secondary NMDA-EPSCs was 174 ± 6% of control (F(1,19) = 6.88; p < .016). A similar upward shift of the secondary response in the morphine group was also observed at 200 ms, because the response was significantly boosted to 123 ± 6% of control (F(1,19) = 14.94; p < .0012). However, the paired-pulse inhibition at 400 ms was not significantly altered between the untreated and morphine groups (F(1,16) = 1.87; p = .19).

Discussion

In previous studies carried out on NAcc slices of naive untreated rats, we reported that NMDA receptor-mediated neurotransmission was under the control of both µ-opioid and metabotropic glutamate receptors (Martin et al., 1997a, b). More specifically, we found evidence that glutamate release was strongly attenuated by agonists selective for µ-opiate receptors and group 2 mGluR receptors located presynaptically. In contrast, the receptors expressed at the postsynaptic level have a different pattern of effects: a µ-opiate agonist enhanced the current evoked by exogenous NMDA, whereas i-AP4, a specific agonist of the group 3 mGluR subtype, decreased it. In the present study, we found that chronic treatment with morphine, known to act on µ-opiate receptors, up-regulated the presumed presynaptic metabotropic depression of NMDA-EPSCs in NAcc neurons but had no influence on the postsynaptic depression of these EPSCs by i-AP4.

Chronic morphine also decreased paired-pulse facilitation of these EPSCs. In the past, paired-pulse facilitation of synaptic potentials has been used as an indirect test of the pre- or postsynaptic site of drug action. Several reports have suggested that there is an inverse relationship between transmitter release and changes in paired-pulse facilitation (Bonci and Williams, 1997; Menenrick and Zorumski, 1995; Salin et al., 1996). This relationship would suggest that chronic morphine further decreases glutamate release in NAcc, because paired-pulse facilitation of NMDA-EPSCs increased after chronic morphine treatment. Therefore, this finding is consistent with our data suggesting up-regulation of pre- but not postsynaptic metabotropic glutamate receptors that depress NMDA-EPSCs. However, it also should be noted that a recent study has found that the magnitude of paired-pulse facilitation of hippocampal CA1 non-NMDA-EPSCs can also be regulated by postsynaptic mechanisms (Wang and Kelly, 1996, 1997).

Mechanisms Underlying Effects of Chronic Morphine. The question arises as to the mechanisms responsible for the chronic morphine effect. At least two mechanisms could be responsible for our findings. First, it is possible that voltage-dependent Ca⁺⁺ channels (VDCC) are involved, because they are known to control the release of neurotransmitters. In fact it has been reported that group 2 mGluR agonists decrease Ca⁺⁺ currents in isolated cortical neurons (Choi and Lovinger, 1996), cerebellar granule cells (Chavis et al., 1995), and striatal neurons (Stefani et al., 1994). Interestingly, Glaum and Miller (1995) reported that this effect observed in isolated cells could also be exerted at the level of presynaptic terminals. The metabotropic glutamate receptor agonist (2S,3S,4S)-α-(carboxycyclopropyl)glycine decreased Ca⁺⁺ currents of neurons of the tractus solitarius, and this effect was inhibited by cyclic GMP-dependent protein kinase inhibitors. Interestingly, it has been shown that voltage-dependent Ca⁺⁺ channels are regulated by adenylyl cyclase, the enzyme known to be linked to the group 2 mGluRs (Pin and Duvoisin, 1995).

This interaction between mGluRs and VDCCs through protein kinase pathways suggests that chronic morphine treatment may increase the inhibitory effect of trans-ACPD on Ca⁺⁺ currents flowing through presynaptic VDCCs and therefore decrease the amount of glutamate released into the synaptic cleft. However, this assumes that the decrease of forskolin-stimulated cAMP formation elicited by the group 2 mGluR agonists (Pin and Duvoisin, 1995) is strengthened after chronic morphine treatment, and that this effect is not counteracted by an increase of cAMP levels induced by other receptors. It has been shown that although chronic morphine treatment down-regulates µ-opiate receptors in the locus ceruleus, it increases cAMP levels (Kogan et al., 1992; Nestler et al., 1993). This effect is viewed as a compensatory mechanism leading to tolerance (Nestler et al., 1993). Although this phenomena, also observed in the NAcc, could run counter to the presumed cAMP decrease hypothesized to inhibit Ca⁺⁺ entry and glutamate release, it is possible that the observation of cAMP up-regulation may apply only to the neuronal somata and mask an opposite effect in the terminals. Indeed, cAMP regulation of the terminals would be dependent not on the NAcc neurons per se, but rather on the afferents whose soma are located either in the prefrontal cortex or the thalamus, structures that have not been shown to present the same cAMP up-regulation as the locus ceruleus and NAcc.

Another possibility involves a mechanism independent of the entry of Ca⁺⁺ that would take place downstream from the VDCC. Thus, metabotropic glutamate agonists like trans-ACPD and (2S,2R,3R)-2-(2-3-dicarboxycyclopropyl)glycine increase the interval between miniature EPSCs isolated in presence of Na⁺ and Ca⁺⁺ channel blockers in the stratum (Tyler and Lovinger, 1995). (2S,2R,3R)-2-(2-3-dicarboxycyclopropyl)glycine is a specific agonist of the group 2 mGluR subtype that is expressed presynaptically in the NAcc. Ca⁺⁺ independence of mGluR effects has been confirmed for CA3 hippocampal neurons, in which 1S,3R-ACPD can decrease glutamate release despite the presence of Cd⁺⁺ (Scanziani et al., 1995).

A good candidate for control of glutamate release that might be directly associated with mGluRs is synapsin I, a phosphoprotein thought to bind to the cytoskeleton and to be partially responsible for regulation of neurotransmitter release (Greengard et al., 1993). Interestingly, it has been shown that this process is under the control of Ca⁺⁺/calmodulin-dependent but also cAMP-dependent protein kinases, and that messenger RNA expression of synapsin I increases markedly after a chronic morphine treatment in various brain regions such as cortex-amygdala, locus ceruleus, and spinal cord (Matus-Leibovitch et al., 1995).
Relevance to Behavioral Effects of Chronic Opiates. The chronic treatment model used here was reported to lead to dependence and tolerance by 12 h postimplantation and to induce withdrawal symptoms up to 13 days after implantation of the pellets (Gold et al. 1994). Our combined data with mGluR agonists and paired-pulse facilitation suggest that this chronic morphine treatment may reduce glutamate release in the NAcc core. Interestingly, this action should decrease NAcc neuronal excitability. It is noteworthy that a similar decreased excitability should occur in the ventral tegmental area, where γ-aminobutyric acid release has been found to increase with chronic morphine (Bonci and Williams, 1997). Furthermore, as noted in the introduction, behavioral experiments have suggested that changes in both mGluRs and NMDA receptors might be responsible for some aspects of opiate tolerance and dependence (Trujillo and Akil, 1991; Fundytus and Coderre, 1997; Fundytus et al., 1997). In addition, the NAcc is thought to play a major role in opiate-seeking behavior or the rewarding properties of opiates. To date, the cellular mechanisms responsible for these phenomena remain unknown. The present studies were undertaken to determine whether the interactions of mGluRs with glutamate release and NMDA receptor function might provide a cellular substrate for some aspects of chronic morphine exposure such as tolerance and dependence. Our previous studies provided data suggesting that activation of NMDA receptors is under the complex, balanced control of both pre- and postsynaptic μ-opioid receptors, and we hypothesized that a disruption of this balance occurred with chronic opiate treatment, perhaps underlying the phenomenon of dependence. Because the presynaptic but not the postsynaptic mGluR regulation of NMDA neurotransmission is enhanced with chronic morphine, our present findings further suggest that chronic morphine treatment also may presynaptically unbalance metabolic regulation of glutamatergic function in the accumbens.

Acknowledgments. We thank Zhiguo Nie and Sam Madamba for technical assistance; J. Netzeband, S. Madamba, and G. Kraus for valuable comments and suggestions on this manuscript, and Dr. Paul L. Herrling (Novartis Pharma LTD) for gifts of several drugs used in this study.