Mu Opiates Inhibit Long-Term Potentiation Induction in the Spinal Cord Slice

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Terman, Gregory W., Clifford L. Eastman, and Charles Chavkin. Mu opiates inhibit long-term potentiation induction in the spinal cord slice. J Neurophysiol 85: 485–494, 2001. Long-term potentiation (LTP) involves a prolonged increase in neuronal excitability following repeated afferent input. This phenomenon has been extensively studied in the hippocampus as a model of learning and memory. Similar long-term increases in neuronal responses have been reported in the dorsal horn of the spinal cord following intense primary afferent stimulation. In these studies, we utilized the spinal cord slice preparation to examine effects of the potent antinociceptive mu opioids in modulating primary afferent/dorsal horn neurotransmission as well as LTP of such transmission. Transverse slices were made from the lumbar spinal cord of 10- to 17-day-old rats, placed in a recording chamber, and perfused with artificial cerebrospinal fluid also containing bicuculline (10 μM) and strychnine (1 μM). Primary afferent activation was achieved in the spinal slice by electrical stimulation of the dorsal root (DR) or the tract of Lissauer (LT) which is known to contain a high percentage of small diameter fibers likely to transmit nociception. Consistent with this anatomy, response latencies of LT-evoked field potentials in the dorsal horn were considerably slower than the response latencies of DR-evoked potentials. Only LT-evoked field potentials were found to be reliably inhibited by the mu opioid receptor agonist Δ-Ala², N-Me-Phe⁴, Gly⁵ enkephalin-ol (DAMGO, 1 μM), although evoked potentials from both DR and LT were blocked by the AMPA/kainate glutamate receptor antagonist 6-cyano-7-nitroquinolinoxide-2,3-dione. Moreover repeated stimulation of LT produced LTP of LT- but not DR-evoked potentials. In contrast, repeated stimulation of DR showed no reliable LTP. LTP of LT-evoked potentials depended on N-methyl-D-aspartate (NMDA) receptor activity, in that it was attenuated by the NMDA antagonist APV. Moreover, such LTP was inhibited by DAMGO interfering with LTP induction mechanisms. Finally, in whole cell voltage-clamp studies of Lamina I neurons, DAMGO inhibited excitatory postsynaptic current (EPSC) response amplitudes from LT stimulation-evoked excitatory amino acid release but not from glutamate puffed onto the cell and increased paired-pulse facilitation of EPSCs evoked by LT stimulation. These studies suggest that mu opioids exert their inhibitory effects presynaptically, likely through the inhibition of glutamate release from primary afferent terminals, and thereby inhibit the induction of LTP in the spinal dorsal horn.

INTRODUCTION

The CNS has been found capable of remarkable plasticity, demonstrating functional and structural changes as a result of incoming stimuli, which then alter the response of that neural system to future stimuli. Such neuroplasticity may play a role in such diverse phenomena as neural protection and regeneration following stroke, epileptogenesis, opiate tolerance, learning and memory, and certain chronic pain states (e.g., Dobkin 1993). The best-studied model of activity-dependent synaptic plasticity, called long-term potentiation (LTP), involves a prolonged increase in neuronal excitation following repeated afferent input to that neuron and has been suggested to be a cellular basis for learning (Bliss and Collingridge 1993). LTP has been most extensively studied in the hippocampus due to the role this region is thought to play in learning and memory and to the highly structured cytoarchitecture of the hippocampus that permits selective afferent stimulation not only in vivo but also in vitro using slice preparations. However, LTP-like phenomena have also been reported in other regions of the neuraxis, including the dorsal horn of the spinal cord in neural circuits likely to mediate nociception (e.g., Randic et al. 1993; Woolf et al. 1989).

For nearly 50 years (Hardy et al. 1952; Mendell 1966) increased pain responses following tissue injury have been hypothesized to occur in part due to changes that occur in the spinal cord dorsal horn. These changes have collectively been termed central sensitization and may last hours to days after cessation of or anesthetic blockade of a repeated nociceptive stimulus (e.g., Codere and Katz 1997; Woolf 1983). Central sensitization has now been demonstrated using a number of response end points (e.g., stimulus threshold, response frequency, receptor field size) and eliciting stimuli (e.g., noxious heat, noxious chemicals, acute joint inflammation and C-fiber electrical stimulation) [for review, see Codere 1993 No. 5 (Baranauskas and Nistri 1998)]. Moreover, a number of studies have indicated that this “central sensitization” in pain transmitting systems may have clinical significance (e.g., Woolf and Doublieu 1994). Intraspinal opioids, for example (Katz et al. 1992), given prior to surgical incision have been reported to reduce postoperative pain and analgesic requirements more than the same dose of drug given after incision—presumably after central sensitization has been induced.

Results of many studies of central sensitization show similarities to studies of LTP in the hippocampus. For example, N-methyl-D-aspartate (NMDA) receptor antagonists have been
reported to block both hippocampal LTP (Harris et al. 1984) and a number of electrophysiological (Liu and Sandkühler 1998; Woolf and Thompson 1991) and behavioral (Coderre and Melzack 1992; Mao et al. 1992; Ren et al. 1992; Seltzer et al. 1991) models of central sensitization. However, since the mechanisms underlying LTP have been shown to differ dramatically from region to region, even within the hippocampus (including NMDA dependence) (Johnston et al. 1992), detailed studies of nociceptive transmission and neuroplasticity within the spinal cord will likely be required to elucidate the mechanisms modulating spinal sensitization.

In the last few years, a number of in vitro studies of long-lasting spinal dorsal horn synaptic plasticity have been published, investigating the inducing stimuli, modulatory pharmacology, and cellular or molecular mechanisms activated during sensitization (e.g., Garraway et al. 1997; Hori et al. 1996; Pickett 1995; Randic et al. 1993; Terman and Chavkin 1997). In this paper, we describe a reliable model of LTP in the neonatal rat spinal cord slice preparation, characterize its dependence on both NMDA and non-NMDA glutamate receptors, and report its modulation by mu opioids acting on LTP induction mechanisms, likely at presynaptic sites on primary afferents. Portions of this data have already been presented in abstract form (Terman and Chavkin 1997).

METHODS

Ten- to 17-day-old rats were anesthetized with halothane, and a laminectomy was performed from mid-thoracic to low lumbar levels. Cold buffer [(in mM) 220 sucrose, 3 KCl, 8 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 d-glucose bubbled with 95% O₂-5% CO₂] was placed on the spinal cord, decapitation was performed, and the cord quickly removed and placed in 35°C, 2% low melting point agar. The placed dorsal root remnants were transferred to an O₂-bubbled, artificial cerebrospinal fluid (ACSF) [(in mM) 113 NaCl, 3 KCl, 1 NaH₂PO₄, 25 NaHCO₃, 11 glucose, 1 CaCl₂, and 2 MgCl₂ plus bicuculline (10 μM) and strychnine (1 μM)] at a rate of 1 ml/min for 1 h prior to electrophysiological recordings.

With the aid of a dissecting microscope, a 3 M NaCl containing glass recording electrode (approximately 5–10 MΩ) was advanced into the medial region of the base of the ipsilateral spinal dorsal horn (Fig. 1). Additionally, one suction electrode (AM Systems) was attached to the dorsal root remnant and another was placed in the tract of Lissauer (LT electrode). In this paper, we describe a reliable model of LTP in the neonatal rat spinal cord slice preparation, characterize its dependence on both NMDA and non-NMDA glutamate receptors, and report its modulation by mu opioids acting on LTP induction mechanisms, likely at presynaptic sites on primary afferents. Portions of this data have already been presented in abstract form (Terman and Chavkin 1997).

As described in the preceding text, the spinal slice preparation was identical to that detailed in the preceding text, with the aid of a dissecting microscope, a 3 M NaCl containing glass recording electrode (approximately 5–10 MΩ) was advanced into the medial region of the base of the ipsilateral spinal dorsal horn (Fig. 1). Additionally, one suction electrode (AM Systems) was attached to the dorsal root remnant and another was placed in the tract of Lissauer (the white matter capping the dorsal horn). This latter electrode was aimed at the tract of Lissauer’s most ventrolateral border to minimize current spread to the dorsal root and the recording site.

FIG. 1. Spinal slice preparation. With the aid of a microscope, a suction electrode is attached to the dorsal root remnant (DR electrode) and another suction electrode is placed in the tract of Lissauer (LT electrode). In this example and in many other slices, LT is difficult to visualize “capping” the dorsal horn, and the LT electrode was placed in the most dorsolateral white matter discernible, lateral to the dorsal horn (as shown). Slices with DR remnants longer than that shown here (for illustrative purposes) were more likely to yield DR field potentials temporally distinct from the stimulus artifact.

Whole cell voltage-clamp studies

Slice preparation was identical to that detailed in the preceding text, but slices were held in place in the experimental chamber with a harp constructed of platinum wire in the shape of a “U” with nylon threads glued across the open end of the U at intervals smaller than the spinal cord diameter. A Zeiss Axioskop FS microscope with a 770 (±40)-nm long-pass filter was used to identify specific cells from which to record. A glass recording electrode [approximately 15 MΩ when filled with (in mM) 125 K₂SO₄, 8 NaCl, 10 HEPES, 2 MgATP, 0.5 NaGTP, and 5 EGTA at pH 7.3 and approximately 285 mOsM] was advanced using a micro-manipulator (Sutter), a CCD camera (Cohu) and a black and white monitor (Sony) until whole cell configuration was obtained using suction on the targeted cell. Whole cell voltage clamp was maintained at a resting potential of −70 mV except as dictated by the particular experiment using an Axopatch 200 amplifier (Axon Instruments) and pclamp 6.0 software (Axon Instruments). Initial series resistance and capacitance were noted and a change of greater than 20% was used as the upper limit for inclusion of data in analysis. Data analysis of peak postsynaptic currents was performed using pclamp 6.0 software and statistical software as in the preceding text.

In glutamate puff experiments, in addition to the recording pipette, a second pipette, containing 100 mM glutamate (Sigma), was placed beginning 30 min after tetanic stimulation compared with the mean response to five test pulses given immediately prior to the 10-Hz stimulation (Baseline). Thus % Potentiation = [(post-tetanus amplitude/baseline amplitude) − 1]*100.

Drug effects were studied at equilibrium from 10 to 15 min after addition of drug to the ACSF perfusate. The mean field potential amplitude of five S1/2 intensity stimuli given immediately prior to drug application was compared with the mean response to five stimuli given beginning 10 min postdrug. Some data were transformed to % Baseline Amplitude [(post-treatment mean amplitude/mean baseline amplitude)*100] and some to % Amplitude Change [(postdrug – predrug mean amplitude)/predrug mean amplitude]*100) for illustrative purposes and statistically analyzed using between subjects and/or within subjects analyses of variance as appropriate (Statistica software) with Tukey’s tests for between group post hoc comparisons. A probability of greater than 0.05 was chosen for statistically significant rejection of the null hypothesis.

Quantitation of responses

A digitizing oscilloscope (Tektronix, Beaverton, OR) was used to measure stimulus-evoked peak-to-peak response amplitudes recorded using an Axopatch 200 amplifier (Axon), and the recording electrode was advanced to maximize these responses. The dorsal root (DR) and the Lissauer’s tract (LT) electrodes were sequentially stimulated (Grass S88) once every minute with a 0.5-ms pulse at the current intensity which produced an approximately half-maximal peak field potential amplitude (S1/2).

In LTP experiments, 12 1-s trains of 10-Hz, 0.5-ms pulses at the S1/2 intensity, given 1 train every 10 s were applied to the specified site. LTP was operationally defined as the mean change in field potential response amplitude for five S1/2 intensity stimuli given
near the voltage-clamped cell. Tract of Lissauer stimulation was alternated with pressure ejection (Picospritzer, General Valve, Fairfield, NJ; 20–40 psi, 50 ms) every 2 min and the evoked responses (EPSCs) recorded.

In paired-pulse experiments, 30 paired (60 ms interstimulus interval) LT electrical stimuli were delivered at 30-s intervals during baseline, [α-Ala², N-Me-Phe², Gly⁵] enkephalin-ol (DAMGO, 1 μM) perfusion, and DAMGO plus naloxone (1 μM) treatments. Paired evoked EPSC amplitudes were converted to a ratio \([P2/P1 = (2\text{nd peak response amplitude})/(1\text{st peak response amplitude})]\) for each stimulus pair, and these were averaged for each treatment for each cell studied. A Friedman test was used for statistical analysis of these repeated measure ratios.

Materials

Bicuculline methiodide, strychnine, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and \(\beta\)-2-amino-5-phosphonovalerate (APV) were purchased from Sigma Chemical. DAMGO was purchased from Research Biochemicals. Naloxone was a gift from NIDA. All drugs were added to the perfusate in a 1:1000 dilution.

RESULTS

Previous studies by others have demonstrated LTP of field potentials in the spinal dorsal horn in vivo (Liu and Sandkühler 1995) and in vitro (Pockett 1995). Our initial studies were directed at pharmacologically characterizing the field potentials elicited by stimulation of LT and DR in spinal cord slices. Studies of central sensitization in the spinal cord have generally found induction of this phenomenon to be particularly dependent on repetitive small fiber activation (e.g., Liu and Sandkühler 1997). LT is a rostrocaudally extending fiber bundle that covers the dorsal horn (also called the dorsal root entry zone) and is known to primarily contain small-diameter primary afferents en route to the dorsal horn where they synapse on intrinsic spinal neurons, particularly those involved in nociception (Chung et al. 1979). Because electrical stimulation thresholds for large fibers are lower than those of small fibers, we hypothesized that there might be differences between evoked potentials from LT and DR stimulation.

Characterization DR and LT stimulation-evoked field potentials

DR and the LT electrodes were stimulated once every minute in alternating sequence with a 0.5-ms pulse at the current intensity that produced a half-maximal field potential amplitude (S1/2). In the presence of bicuculline (10 μM) and strychnine (1 μM), inhibitors of GABA\(_A\) and glycine inhibitory neurotransmission, respectively, both LT and DR stimulation produced presumably excitatory field potentials whose amplitudes were stable for many minutes (see Fig. 3A for example and Fig. 2A for group means). Both of these evoked potentials were significantly inhibited by 10 mM MgCl\(_2\) (Fig. 2A), suggesting that the potentials represent a synthetically mediated response. Moreover, the AMPA and kainate glutamate receptor antagonist CNQX (10 μM) also inhibited both LT and DR potentials (Fig. 2A), implicating excitatory amino acids in mediating both of these excitatory responses [both AMPA (Willocockson et al. 1984a) and kainate (Li et al. 1999) glutamate receptors have been implicated in mediating dorsal horn neurotransmission]. LT- and DR-evoked potentials did differ from each other, however, in apparent response latency. At identical stimulus intensities, DR produced a field potential with an estimated minimum conduction velocity of 3–5 m/s (calculated by the distance between the stimulating electrode and the recording electrode divided by the latency of the initial field potential peak; \(n = 4\)), whereas the LT-evoked field potentials had an approximately 0.5–1 m/s minimum conduction velocity \((n = 4)\), suggesting, as expected, that smaller fibers may be involved in mediating the LT-evoked responses. The DR- and LT-evoked field potentials also differed from one another in their modulation by mu opioids. The LT- but not the DR-, evoked response was significantly inhibited by the mu agonist DAMGO (1 μM; \(n = 6\); Fig. 2B), perhaps indicative of the greater proportion of LT fibers thought to be involved in nociception in comparison to the DR (Coggeshall et al. 1981). The opioid antagonist naloxone (1 μM) significantly reversed the DAMGO inhibition of LT-evoked responses without having any effect by itself (Fig. 2B).

LT field potentials (e.g., Fig. 3A, inset) were significantly potentiated 30 min after 10-Hz tetanic stimulation (12 1-s trains of 10-Hz 0.5-ms pulses at the S1/2 intensity, given 1 train every 10 s) of LT (Fig. 3, A and B) but not DR (Fig. 3B). Tetanic stimulation of DR (using the same stimulus parameters) produced no significant potentiation of either LT- or DR-evoked potentials (Fig. 3B).

Pharmacological modulation of LTP of LT-evoked responses

LTP was operationally defined in these studies as the potentiation of LT-evoked field potentials 30 min following tetanic stimulation. This time period is longer than those employed in other distinct models of activity-dependent potentiation including post-tetanic potentiation and short-term potentiation and has been utilized by ourselves and others (e.g., Alzheimer et al. 1991; Terman et al. 1994) in studies of hippocampal LTP. LTP was also noted at 1 h and 90 min post LTP induction in our initial studies of spinal LTP. However, since determining the time course of LTP was not a goal of these experiments, these time points were examined in only a few cells.

Nociceptive sensitization, as mentioned in the preceding text, has been linked to activation of the glutamate NMDA receptor in that NMDA antagonists can prevent sensitization in vivo and in vitro. We studied the role of NMDA receptors in the induction of LT-evoked response LTP. Pretreatment of slices for 10 min with the NMDA antagonist APV (50 μM) had no effect on LT-evoked response amplitudes (data not shown) but significantly inhibited LTP following tetanic LT stimulation (Fig. 4A). This LTP was also inhibited by pretreatment with DAMGO (1 μM; Fig. 4A), an effect antagonized by co-administration of naloxone (1 μM). Naloxone itself, had no effect on LTP (Fig. 4A), suggesting that endogenous opioids have no role in modulating this phenomenon.

The inhibitory effect of mu opioids on LTP produced by repeated LT stimulation could conceivably be due to effects on LTP induction, expression, or maintenance mechanisms or some combination of these three. To determine which of these mechanisms of LTP are primarily inhibited by DAMGO, we administered DAMGO to three separate groups of slices, followed 10 min later by naloxone. The timing of the DAMGO and naloxone administration differed between groups with respect to the 2-min LTP-inducing stimulus (LT tetanic stim-
ulation). In one group, both DAMGO and naloxone were given 10 min before induction. In another group, DAMGO was given before LTP induction and naloxone was given after it. In the third group of slices, both DAMGO and naloxone were given immediately after the LTP-inducing stimulus.

LTP was inhibited only when DAMGO was bound to opiate receptors (i.e., naloxone was not present) during LTP induction (Fig. 4B), suggesting that it is induction mechanisms with which DAMGO interferes predominantly. The inhibition of induction noted here does not rule out additional mu effects on maintenance and, particularly, expression mechanisms of LTP. Indeed, DAMGO seemed to inhibit to some degree the expression of potentiation prior to naloxone administration when both DAMGO and naloxone were given after LTP induction. Nonetheless data were not routinely collected at this short interval after LTP induction in this or other groups making comparisons impossible. We can conclude, however, that effects on induction are sufficient to explain the inhibition of LTP by mu opioids.

**DAMGO effects on LT-evoked EPSCs**

The inhibitory effect of DAMGO on LTP induction could be due to either presynaptic or postsynaptic effects of the opioid agonist. Both pre- (e.g., Hori et al. 1992; Kangra and Randic 1991) and postsynaptic (e.g., Jefetinija 1988; Yoshimura and North 1983) effects of mu opioids on spinal neurons have been reported previously. Indeed in our studies using evoked field potentials as a measure of neural activity, both pre- and postsynaptic effects might occur at the same or different synapses monitored by our recording electrode. To differentiate such effects, we utilized the whole cell voltage-clamp technique to record from specific dorsal horn cells activated synaptically by LT stimulation.

For these studies, cells in Lamina I of the dorsal horn were chosen because of the known importance of this cell layer in relaying nociceptive information from primary afferents to supraspinal sites (Lima et al. 1993) [e.g., nociceptive specific spinohalamic cells are most common in this spinal cord layer (Willis and Westlund 1997)]. As demonstrated above with LT stimulation-evoked field potentials, LT stimulation-evoked postsynaptic currents (EPSCs) were also consistently inhibited by DAMGO (see Fig. 5 for example and Fig. 6B for pooled data) in a naloxone reversible manner. Again, in the presence of bicuculline and strychnine, CNQX almost completely eliminated residual postsynaptic currents (Figs. 5 and 6B) as it had
the field potentials. In these studies, however, the effects of bicuculline and strychnine were also examined prior to DAMGO application. Bicuculline and strychnine were both administered to all cells to block GABA$_A$ and glycine-mediated inhibitory postsynaptic currents (IPSCs) respectively, as before. However, unlike in previous studies, the two antagonists were added one at a time in a counterbalanced fashion. All cells were found to have LT activated bicuculline- or strychnine-sensitive synaptic inputs (the combination of both drugs inhibited PSC amplitudes by 48 ± 14%, mean ± SD; n = 17; data not shown). A significant inhibition of PSC amplitudes was produced only when strychnine (n = 9) was added first to the perfusate (Fig. 6A), although bicuculline also had inhibitory effects in many cells independent of the effects of strychnine on that cell. The activation of GABA and glycine-mediated IPSCs in Lamina I neurons by LT stimulation parallels results of others using other primary afferent stimulation or in other dorsal horn laminae (e.g., Yoshimura and Nishi 1993). In the remainder of the studies, bicuculline and strychnine were routinely added to the perfusate prior to any recording as in the field potential studies.

Presynaptic inhibition of LT-evoked EPSCs by DAMGO

In all cells perfused to date with DAMGO under whole cell voltage-clamp conditions, current/voltage (I-V) curves were constructed prior to and following DAMGO administration. No naloxone reversible changes in current amplitudes at any holding potentials (200-ms, 20-mV steps from −120 to 20 mV) have been observed in any cell (n = 22; e.g., Fig. 7A).

The absence of effects of DAMGO on I-V curves in voltage-clamped Lamina I neurons, suggests that the DAMGO effects are presynaptic rather than postsynaptic in this region, presumably acting on mu receptors located on primary afferent terminals to inhibit neurotransmission and perhaps LTP. We also evaluated possible postsynaptic DAMGO inhibitory effects in
this region by comparing, in the same voltage-clamped cell, DAMGO's effects on LT stimulation evoked EPSCs and on excitatory currents evoked by puffing exogenous glutamate near the cell. As before, LT stimulation evoked CNQX-sensitive EPSC amplitudes were inhibited by DAMGO (by 37 ± 11%, n = 10), in a naloxone-reversible way (e.g., Fig. 7B). However, the amplitude of currents produced by glutamate puffs were not significantly inhibited by DAMGO (decreased 8 ± 9%, n = 10), although they were significantly attenuated by CNQX (by 72 ± 17%). These data give further evidence for a primarily presynaptic inhibitory effect of DAMGO on excitatory transmission in Lamina I of the spinal dorsal horn, probably via inhibition of glutamate release from primary afferent terminals—providing a likely mechanism for DAMGO’s inhibition of LTP induction seen in our field potential studies.

The effect of DAMGO on glutamate release was further evaluated using a paired-pulse stimulation paradigm—a common technique utilized to assess the pre- or postsynaptic effects of a neuromodulator (e.g., Simmons et al. 1994). When pairs of stimuli are delivered in rapid succession, residual calcium in the presynaptic terminal following the first stimulation leads to an enhanced transmitter release and a resultant facilitated postsynaptic response to the second stimulus (e.g., Debanne et al. 1996). Paired-pulse facilitation is enhanced when the probability of neurotransmitter release is relatively low, and thus treatment-induced alterations in paired-pulse facilitation reflect changes in the probability of neurotransmitter release from the presynaptic terminal.

Paired-pulse facilitation of LT stimulation-evoked EPSCs was significantly potentiated in Lamina I neurons following DAMGO (1 μM) application (Fig. 8). This effect was reversed by naloxone (1 μM), and both of the paired responses were virtually eliminated by CNQX (10 μM; data not shown) supporting the conclusion that DAMGO acts in this region to decrease the release of glutamate from presynaptic terminals.

**DISCUSSION**

Much evidence suggests that neurotransmission in the spinal dorsal horn, as in other areas of the CNS, is capable of...
tremendous activity-dependent plasticity that can cause long-term consequences for subsequent responses to stimuli and thereby sensitize nociceptive responses and perhaps lead to certain chronic pain states. In this manuscript, we report studies of in vitro spinal dorsal horn neurotransmission and LTP following tetanic stimulation of small-diameter primary afferents. We find that LTP but not pretetanus neurotransmission is dependent on NMDA receptors and that both neurotransmission and LTP induction can be inhibited by mu opioid analgesics. In individual dorsal horn cells, mu opioids inhibit glutamate-mediated neurotransmission from primary afferents independent of effects on the postsynaptic cells. This suggests that presynaptic inhibition of glutamate release may be sufficient to explain the inhibitory effects of mu opioids on neurotransmission and LTP induction.

Our findings of reliable and reproducible evoked field potentials in the rat dorsal horn and LTP of these responses following repeated primary afferent stimulation have also been reported by others (Liu and Sandkuhler 1995, 1997, 1998; Liu et al. 1997; Pockett 1995; Sandkuhler and Liu 1998). In our studies, electrical stimulation of Lissauer’s tract is particularly effective in producing LTP in comparison to dorsal root stimulation. Although it is possible that this difference is due to an LTP inhibitory mechanism (relying neither on glycine nor GABA_A substrates) activated by dorsal root stimulation, our response latency estimates suggest that we are simply stimulating different sized primary afferents at the two stimulation sites. Admittedly, the inherent uncertainty in our studies concerning current spread of the stimulation and the location of neural processes contributing to the field potentials makes it impossible to compare our estimates of conduction velocity to those of primary afferents known to mediate nociception in vivo (C and Aδ fibers). Moreover decreases in the degree of myelination in neonatal rat spinal cord compared with the adult

![Image](http://jn.physiology.org/)

**FIG. 7.** Mu opioid inhibition of LT evoked Lamina I EPSCs at a presynaptic site. A: membrane currents recorded in whole cell voltage-clamp during a series of 200-ms voltage steps (from a −70 mV holding potential) performed immediately prior to DAMGO (pre-drug control), 10 min following DAMGO (1 μM), and 10 min following naloxone (1 μM; DAMGO + naloxone; n = 10). No significant naloxone reversible changes in current amplitudes [DAMGO − (DAMGO + naloxone)] were observed at any potential. B: DAMGO (1 μM) inhibited LT stimulation-evoked EPSC amplitudes in a naloxone (1 μM) reversible way. In contrast, glutamate puffed (20–40 psi, 50 ms) from a glass electrode placed near the cell, evoked excitatory currents that were not inhibited by DAMGO but were inhibited by CNQX (10 μM), verifying that the observed currents were glutamate mediated. This experiment was repeated in 10 cells. As in this example, LT stimulation evoked CNQX-sensitive EPSC amplitudes that were significantly inhibited by DAMGO (37 ± 11%). In contrast, amplitudes of currents produced by glutamate puffs were not significantly inhibited by DAMGO (decreased 8 ± 9%) although these currents were significantly attenuated by CNQX (by 72 ± 17%).

**FIG. 6.** Neuropharmacology of LT stimulation evoked postsynaptic currents (PSCs) in Lamina I. A: effects of strychnine (1 μM) or bicuculline (10 μM) on Lamina I PSCs. Only strychnine (n = 9) produced a significant inhibition of PSC amplitudes although bicuculline (n = 8) also seemed to have inhibitory effects in some cells. The combination of both drugs (n = 17) inhibited PSC amplitudes by 48 ± 14% (data not shown). B: following addition of both bicuculline and strychnine to the perfusate, DAMGO (1 μM for 10 min) significantly inhibited EPSCs. This inhibition was reversed by naloxone (1 μM). CNQX (10 μM) was also found to significantly inhibit these EPSCs, suggesting a role of AMPA and/or kainate glutamate receptors in mediating these responses. Graphed data are from 8 cells. *Significant difference from the mean amplitudes immediately prior to drug administration (baseline) (note that the same cell has different baseline values for A and B).
inducing sensitization (Baranauskas and Nistri 1998) and in their sensitivity to mu opioids [as seen in the present studies and those of Li et al. (1999)].

Many in vitro (e.g., King and Thompson 1989; Li et al. 1999; Schneider et al. 1998) and in vivo (e.g., Dougherty and Willis 1991; Headley et al. 1987; Procter et al. 1998; Radhakrishnan and Henry 1993; Willcockson et al. 1984a) studies of primary afferent-dorsal horn neurotransmission have identified an important role of glutamate. Indeed, glutamate has been found to excite nearly 100% of dorsal horn neurons thought to be involved in pain processing, primarily via AMPA (Dougherty and Willis 1991; Headley et al. 1987; Willcockson et al. 1984a) and, more recently, kainate (Li et al. 1999; Procter et al. 1998) receptors. Thus our finding that the AMPA antagonist CNQX greatly diminished both LT and DR stimulation evoked responses is not surprising. Recently ATP acting at P2X receptors on spinal neurons (dissociated or in culture) has also been implicated in “fast transmission” (Bardoni et al. 1997; Gu and MacDermott 1997) perhaps relevant to nociception (Bland-Ward and Humphrey 1997; Cook et al. 1997) [although this is controversial (Li et al. 1998)]. Whether the very small CNQX-resistant components of evoked potentials and Lamina I EPSCs observed in our studies were mediated by ATP was not specifically examined. However, in the field potential studies, the CNQX-resistant responses were not significantly different from those observed in high magnesium buffer, suggesting that this technique has little sensitivity for studying nonglutamatergic fast synaptic transmission. Such fast transmission is in contrast to slower onset NMDA receptor-mediated glutamate actions within the dorsal horn (King and Lopez-Garcia 1993), which normally follow initial activation of AMPA receptors (Jeftinija and Urban 1994; Randic et al. 1993; Svendsen et al. 1998) and are consistent with a role for NMDA receptors in mediating longer latency plastic changes (as seen in our LTP studies here). Mu opiates have been reported capable of inhibiting both AMPA (Hori et al. 1992) and NMDA (Jeftinija 1988; Willcockson et al. 1984b) receptor-mediated primary afferent neurotransmission in the dorsal horn via both pre- (Hori et al. 1992) and postsynaptic (Jeftinija 1988) mechanisms.

The inhibition of LTP induction by DAMGO and, particularly, its inhibition of effects from synaptically released glutamate (but not exogenous glutamate) suggests that DAMGO acts by inhibiting presynaptic glutamate release. This suggestion is strengthened by our observation of a naloxone-sensitive DAMGO-induced enhancement of paired-pulse facilitation. Others have demonstrated presynaptic opioid effects on excitatory dorsal horn synaptic transmission (Glaum et al. 1994; Hori et al. 1992; Jeftinija 1988; Kohno et al. 1999). For example, Kohno et al. (1999) reported inhibitory effects of DAMGO on glutamatergic EPSCs in Lamina II (substantia gelatinosa) cells. In addition, they found that miniature EPSC frequency was also inhibited, further supporting the hypothesis that these effects were due to a decrease in glutamate release. The molecular mechanisms underlying these effects are not known. Both an inhibition of calcium channels and an activation of potassium channels have been suggested to mediate mu opiate receptor effects in the spinal cord (Duggan and North 1983; Schneider et al. 1998; Schroeder et al. 1991; Taddese et al. 1995). Although we were unable to document any postsynaptic effects of DAMGO, such effects have also been reported in the rat superficial dorsal horn (Yoshimura and North 1983). It is important to note however, that these effects have been most commonly reported in Lamina II cells and not the Lamina I cells studied here. Indeed there appear to be no reports of Lamina I postsynaptic effects, which is again consistent with the related anatomical literature. Although rhizotomy studies have found that half of dorsal horn mu opiate receptors are intrinsic to the spinal cord (Coggshall and Carlton 1997), Cheng et al., using ultrastructural analysis, have localized just 12% of superficial dorsal horn mu receptors to somata or dendrites (Cheng et al. 1997). This suggests that the remaining mu receptors are located on axon terminals of spinal interneurons and concurs with the low incidence of postsynaptic neu-
The presynaptic effects of mu opioids in inhibiting LTP induction in the spinal cord parallel our findings in the dentate region of the hippocampus that kappa opioids inhibit LTP induction (Terman et al. 1994; Wagner et al. 1993). Moreover we have found in the dentate that the endogenous kappa opioids, dynorphins, are released from postsynaptic neurons during repeated afferent stimulation and act presynaptically to modulate LTP (Simmons et al. 1994). No evidence of endogenous opioid effects were unmasked in the present studies by naloxone, although Pockett has previously reported (Pockett 1995) naloxone-induced inhibition of long-term depression of field potentials in the spinal cord. Indeed preliminary evidence from our laboratory suggests that much like the hippocampus, dynorphins in the spinal cord may provide feedback inhibition to modulate LTP. The kappa opioid receptor antagonist norbinaltorphimine, significantly potentiates LTP of Lamina I EPSCs (Taylor et al. 1998). In addition, other mechanisms appear to provide feedback inhibition for LTP in the spinal cord. In a series of studies, Sandkuhler’s group has investigated in vivo the underlying pharmacology and necessary stimulus parameters for inducing dorsal horn field potential LTP evoked by primary afferent stimulation (Liu and Sandkuhler 1995, 1997, 1998; Liu et al. 1998; Sandkuhler and Liu 1998). Although C-fiber stimulation and resultant release of neurokinins have been found to be critical for inducing LTP in their studies, both A-delta primary afferent activity (Liu et al. 1998) and descending inhibitory mechanisms (Sandkuhler and Liu 1998) can powerfully inhibit such LTP.

The importance of spinal LTP in mediating central sensitization reported in laboratory animals and man is not known. A number of other mechanisms have been proposed to account for changes in sensitivity of spinal nociceptive neurons to stimulation. These include decreases in intrinsic (Wiesenfeld-Hallin et al. 1997) or descending inhibition (Sandkuhler and Liu 1998), increases in descending facilitation (and facilitatory) (Urban et al. 1999) and increases in numbers of excitatory synaptic connections (e.g., Woolf and Dou bell 1994; Woolf et al. 1992, 1995). Nonetheless, pretreatment with mu opioids have been found effective in blocking exacerbations of postoperative (Katz et al. 1992) and chronic pain (Bach et al. 1988) and has led to the concept of “preemptive analgesia” (Katz et al. 1992) as a strategy to avoid activity-dependent neuroplastic changes in nociception. The current studies of primary afferent LTP in the spinal cord support the idea of aggressive treatment of nociception with mu opioids in an effort to inhibit LTP induction.

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