Systemic Morphine Inhibits Dorsal Horn Projection Neurons Through Spinal Cholinergic System Independent of Descending Pathways

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ABSTRACT

Cholinergic circuitry and muscarinic receptors within the spinal cord have been proposed to contribute to the analgesic effects of systemic morphine. In this study, we determined if the descending pathways are involved in the inhibitory effect of systemic morphine on dorsal horn projection neurons mediated by activation of the spinal cholinergic system. Single-unit activity of dorsal horn projection neurons was recorded in anesthetized rats. The neuronal responses to mechanical stimuli applied to the receptive field were determined before and after intravenous injection of morphine. The inhibitory effect of intravenous morphine on dorsal horn neurons was also tested before and after topical spinal application of the muscarinic antagonist atropine in both intact and spinally transected rats. Intravenous injection of 2.5 mg/kg morphine significantly inhibited the evoked response of dorsal horn neurons in both intact and spinally transected rats. Spinal topical application of the µ opioid antagonist CTAP completely blocked the effect of morphine on dorsal horn neurons. Also, spinal application of 10 µM atropine significantly attenuated the effect of systemic morphine. In rats subjected to cervical spinal transection, atropine produced a similar attenuation of the inhibitory effect of systemic morphine on dorsal horn neurons. Data from this electrophysiological study suggest that systemic morphine inhibits ascending dorsal horn neurons through stimulation of spinal µ opioid receptors. Furthermore, activation of the local spinal cholinergic circuitry and muscarinic receptors is involved in the inhibitory effect of systemic morphine on dorsal horn projection neurons independent of descending pathways.
INTRODUCTION

The opioid agonist morphine is often administered systemically to treat patients with moderate and severe pain. Morphine produces a potent analgesic action through activation of pre- and postsynaptic µ opioid receptors in the brain and spinal cord dorsal horn (Basbaum and Fields, 1984; Kalyuzhny et al., 1996; Schneider et al., 1998; Kohno et al., 1999). The dorsal horn of the spinal cord is an important site for nociceptive transmission as well as modulation. Systemic and spinal local application of opioids both produce potent analgesia and inhibition of dorsal horn neurons (Yaksh and Rudy, 1977; Sastry and Goh, 1983; Yaksh and Noueihe, 1985; Magnuson and Dickenson, 1991). The spinal dorsal horn neurons subject to descending modulation from supraspinal sites such as the periaqueductal gray, locus coeruleus, and rostral ventromedial medulla (Dostrovsky et al., 1983; Bodnar et al., 1990; Urban and Smith, 1994; Pan et al., 2004). Some studies suggest that the analgesic effect of opioids may be mediated by descending inhibition of dorsal horn neurons (Basbaum and Fields, 1984; Wigdor and Wilcox, 1987; Chiang and Zhuo, 1989). However, systemic morphine appears more effective in increasing the nociceptive threshold when the descending modulation is removed (Sinclair et al., 1988). Furthermore, supraspinal morphine application increases, but not decreases, the evoked response of dorsal horn neurons (Sinclair, 1986). It remains uncertain if and to what extent the inhibitory effect of systemic morphine on dorsal horn neurons involves the descending pathways.

The spinal cholinergic system and muscarinic receptors are closely involved in antinociception produced by systemic morphine. In this regard, intravenous injection of morphine increases the release of acetylcholine in the spinal dorsal horn (Bouaziz et al., 1996;
Xu et al., 1997). Also, spinal endogenous acetylcholine mediates the analgesic effect of systemic morphine primarily through muscarinic receptors (Chen and Pan, 2001). However, the sources of cholinergic neurons that release acetylcholine to inhibit spinal dorsal horn neurons upon systemic morphine are not fully known. Systemic morphine may activate the spinal cholinergic circuitry to inhibit dorsal horn neurons directly or indirectly through descending pathways. Although histological and functional evidence supports intrinsic cholinergic innervation of the spinal cord (Barber et al., 1984; Sherriff et al., 1991; Todd and Spike, 1993; Zhang et al., 2005), some behavioural and microdialysis studies suggest that systemic morphine activates the local spinal cholinergic system indirectly through supraspinal descending pathways (Chiang and Zhuo, 1989; Xu et al., 1997). In the present study, we used neurophysiological techniques to test the hypothesis that systemic morphine inhibits spinal dorsal horn projection neurons through interaction with the spinal cholinergic system and this effect is dependent of supraspinal descending modulation.
MATERIALS AND METHODS

General procedures

Male rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 250-300 g were used in this study. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine (Hershey, PA) and conformed to the NIH guidelines on the ethical use of animals. Anesthesia was initially induced with 2% halothane in 100% oxygen. The left jugular vein and carotid artery were cannulated for intravenous drug administration and blood pressure monitoring, respectively. Following cannulation, sodium pentobarbital (50 mg/kg) was given intravenously and supplemented when necessary. Adequate depth of anesthesia was confirmed by the absence of corneal reflexes, withdrawal reflexes to a noxious stimulus, and spontaneous blood pressure fluctuations. The trachea was cannulated, and the rat was ventilated mechanically using a rodent ventilator. The respirator was adjusted to keep the end-tidal CO$_2$ concentration at 4%, monitored by a Capstar-100 CO$_2$ Analyzer (CWE, Inc., Ardmore, PA). Laminectomies were performed to expose the spinal cord at the C$_{1-3}$ and L$_{2-5}$ levels. Around the exposed lumbar spinal cord, a small pool (approximately 0.2 ml) was formed by the surrounding tissues to serve as a reservoir for topical application of drugs. After the dura was removed at both sites, the spinal cord was covered with artificial cerebrospinal fluid (aCSF) solution.

Single-unit recording of dorsal horn projection neurons

During neuronal recordings, rats were briefly paralyzed with pancuronium bromide (1
mg/kg, i.v.). Between protocols, the effect of pancuronium bromide was allowed to wear off, and the adequacy of anesthesia was verified by the absence of the withdrawal response to tail-pin. Neuromuscular blockade was assessed by electrical stimulation of skeletal muscle contraction through a pair of electrodes inserted into the sural muscle. A separate bipolar metal stimulating electrode was inserted into the ventrolateral quadrant of the spinal cord at the C5-6 segment. Dorsal horn neurons in the contralateral side of the lumbar enlargement were recorded with a glass electrode filled with 5% KCl solution (resistance 4-6 MΩ). A motorized manipulator (David-Kopf Instruments, Tujunga, CA) was used to descend the recording electrode gradually until the single-unit activity of a dorsal horn projecting neuron was recorded (Chen and Pan, 2002; Chen and Pan, 2004). The electrode was inserted no more than 1 mm below the dorsal surface of the spinal cord. Individual ascending dorsal horn neurons in the lumbar enlargement were antidromically identified and characterized, as we described in detail previously (Chen and Pan, 2002). The stimulus was 0.5-1.0 mA, 0.2 ms, and 0.8-1 Hz (S48 Stimulator, Grass Instruments, Quincy, MA). The dorsal horn neurons were considered to be antidromically activated if the following criteria were met: 1) the antidromically evoked spikes occurred at a constant latency; 2) the antidromically evoked spikes followed a high frequency (400 Hz) stimulation; and 3) the antidromic action potential collided with the orthodromic spike within the critical interval. The action potential of the neuron was amplified, filtered with a band-pass filter (DAM 80; World Precision Instruments, Sarasota, FL) and processed through an audioamplifier (model AM9; Grass Instruments, West Warwick, RI) and monitored on a storage oscilloscope (Tektronix Inc., Beaverton, OR). The neuronal activity also was recorded into a computer through an A/D interface board for subsequent off-line quantitative analysis. The
single unit was identified initially by examining the waveform and the spike amplitude on an oscilloscope at a rapid sweep speed as well as the recorded sound frequency related to each spike. Single-unit activity of the dorsal horn neuron was isolated using a software window discriminator (DataWave Technology, Longmont, CO). When an event was detected, the associated waveform (6 ms) was extracted and displayed continuously in a separate software oscilloscope window. Therefore, single unit recording was confirmed by the constancy of the shape and polarity of the displayed spike waveform. Discharge frequency was quantified by using data acquisition and analysis software (Experimental Workbench; DataWave Technology).

A majority of the dorsal horn projection neurons had a receptive field on the glabrous skin of the hindpaw. After the cutaneous receptive field was located and marked, the responses of dorsal horn neurons to the following mechanical stimuli were initially tested as the control (Chen and Pan, 2002; Chen and Pan, 2004). The wooden tip of a cotton-tipped applicator was used to apply the pressure stimulus. The tip was applied perpendicularly to the skin for 6-8 s to generate an intense pressure (~200 g/mm²), which was perceived by the investigator as mildly painful. The pinch stimulus was applied for 6-8 s by means of a small forceps with a strong grip (~560 g/mm²) that produces distinct pain when applied to human skin without causing tissue damage. The pressure and force generated by pressure and stimuli were estimated using a displacement transducer before but not during the experiment. The effect of morphine on the neuronal response to a touch stimulus was not examined because opioids primarily affect the evoked responses to nociceptive stimuli (Hylden and Wilcox, 1986; Khan et al., 2002).
Experimental protocols

1. Role of spinal μ opioid receptors in the effect of systemic morphine on dorsal horn neurons

After recording the baseline activity of the identified dorsal horn projecting neuron for 5 min, the neuronal responses to pressure and pinch applied to the receptive field were examined before, 10 min and 1 hr after morphine (2.5 mg/kg, i.v.). This dose of morphine was chosen because it consistently produces analgesia in unanesthetized rats (Chen and Pan, 2001). To determine if repeat morphine administration produces a similar inhibitory effect on the dorsal horn neuron, morphine (2.5 mg/kg, i.v.) was injected again 1 hr after the initial dose of morphine and when the firing activity and evoked responses of dorsal horn neurons returned to the control. In the pilot study, we observed that the inhibitory effect of morphine on dorsal horn neurons lasted less than 50-60 min and the evoked response of dorsal horn neurons fully recovered 1 hr after intravenous injection of 2.5 mg/kg morphine.

To study the role of spinal μ opioid receptors in the inhibitory effect of systemic morphine, 1 μM H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP), a highly specific μ receptor antagonist) (Kohno et al., 1999), was topically applied to the recording site in the lumbar spinal cord 1 hr following the initial morphine. The responses of the dorsal horn neurons to mechanical stimuli applied to the receptive field were tested 5 min after CTAP application. Subsequently, the evoked response of dorsal horn neurons was tested 10 min after repeat administration of morphine (2.5 mg/kg, i.v.).

2. Role of spinal muscarinic receptors in the inhibitory effect of systemic morphine on
dorsal horn neurons

In this protocol, the inhibitory effect of systemic morphine (2.5 mg/kg, i.v.) on evoked response of dorsal horn projection neurons to mechanical stimuli was tested before spinal application of the specific muscarinic receptor antagonist atropine. Atropine (10 µM, dissolved in artificial cerebrospinal fluid) was topically applied to the recording site (n = 11) of the lumbar spinal cord 5 min before the second dose of morphine was injected. We have shown that 10 µM atropine completely blocks the inhibitory effect of muscarinic receptor agonists on dorsal horn projection neurons (Chen and Pan, 2004). The responses of dorsal horn neurons to press and pinch were tested 10 min after each injection of morphine.

3. Role of descending pathways in stimulation of spinal cholinergic system involved in the inhibitory effect of systemic morphine on dorsal horn neurons

To determine if supraspinal descending pathways are involved in stimulation of spinal cholinergic system to inhibit dorsal horn neurons following systemic morphine, we examined the effect of spinal application of atropine on the inhibitory action of systemic morphine in spinally transected rats. For complete transection of the cervical spinal cord, one small section of spinal cord in the C1-3 cervical region (rostral to the stimulating electrode) was gently lift and cut off. In one protocol, we tested the inhibitory effect of repeat systemic morphine (2.5 mg/kg, i.v.) on dorsal horn projection neurons 30 min after spinal cord transection.

In another protocol, we examined the inhibitory effect of systemic morphine (2.5 mg/kg, i.v.) on dorsal horn projection neurons following spinal topical application of atropine in spinally transected rats. In this protocol, surgical transection was done 30 min after the first dose of
morphine. Atropine (10 µM) was topically applied to the recording site of the lumbar spinal cord 30 min following spinal transection. The second dose of morphine was then injected 5 min after atropine application. The neuronal responses to mechanical stimuli applied to the receptive field were tested 10 min after injection of the second dose of morphine.

Morphine was obtained from Astra Pharmaceuticals (Westborough, MA). Atropine and CTAP were purchased from Sigma (St. Louis, MO). At the end of the experiments, rats were killed by an intravenous injection of an overdose of sodium pentobarbital.

**Data analysis**

Data are presented as means ± S.E.M. The baseline firing rate of the dorsal horn neuron was averaged during 5 min control period. The evoked responses were quantified as the mean discharge rate over the duration of the stimulus after subtracting the background activity of the neuron (Chen and Pan, 2002; Chen and Pan, 2004). Significant changes in the drug effect on evoked responses of dorsal horn neurons to the mechanical stimuli were determined using one-way ANOVA followed by Tukey’s post hoc test. Differences were considered to be statistically significant if P < 0.05.
RESULTS

A total of 46 ascending dorsal horn neurons from 46 rats was studied. All the dorsal horn projection neurons included in this study were wide-dynamic-range neurons, i.e., cells responding to brush but responding more intensely to noxious stimuli (pinch > press). The ascending dorsal horn neurons recorded in the lumbar spinal cord had a mean depth of 652 ± 25 µm, ranging from 370 to 960 µm.

1. Role of spinal µ opioid receptors in the effect of systemic morphine on dorsal horn neurons

Systemic morphine (2.5 mg/kg, i.v.) significantly inhibited the evoked activity of dorsal horn projection neurons in response to press and pinch in all 9 cells tested (Fig. 1). One hr after the initial morphine injection, the baseline activity and the evoked response of the dorsal horn projection neurons completely returned to the control. Repeat injection of the same dose of morphine reproducibly inhibited the evoked response of these 10 dorsal horn neurons (Fig. 1). There was no significant difference in the inhibitory effect on dorsal horn neurons between the initial and second dose of morphine.

In 6 separate dorsal horn projection neurons studied, intravenous morphine (2.5 mg/kg) produced a profound inhibition of the evoked response of these cells. Topical spinal application of the specific µ opioid receptor antagonist CTAP (1 µM) alone had no significant effect on the dorsal horn cells. However, in the presence of 1 µM CTAP, subsequent intravenous injection of 2.5 mg/kg of morphine failed to attenuate the evoked response of dorsal horn neurons (Fig. 2).
2. Role of spinal muscarinic receptors in the inhibitory effect of systemic morphine on dorsal horn neurons

In 11 dorsal horn projection neurons, the first dose of morphine (2.5 mg/kg, i.v.) significantly inhibited the evoked response to pressure and pinch applied to the receptive field. Atropine (10 µM) was applied topically to the recording site of the lumbar spinal cord 1 hr after initial morphine. Atropine alone had no significant effect on the evoked response of these dorsal horn cells (Fig. 3). In the presence of 10 µM atropine, the inhibitory effect of the second dose of morphine on dorsal horn neurons was significantly attenuated, compared to the effect of the first dose of morphine on press- and pinch-evoked responses of the same neurons (Fig. 3).

3. Role of descending pathways in stimulation of spinal cholinergic system involved in the inhibitory effect of systemic morphine on dorsal horn neurons

Before spinal transection, the first dose of intravenous morphine (2.5 mg/kg) significantly inhibited the evoked response of 20 dorsal horn projection neurons (n = 20 rats) to press and pinch (Figs. 4 and 5). The spinal cord was surgically transected 30 min after the initial morphine. The baseline activity in 5 of 20 neurons increased immediately after transection, and the neurons showed increased spontaneous activity from 0.34 ± 0.17 to 3.92 ± 0.33 Hz (P < 0.05) for about 10-15 min. One hr after the first dose of morphine (30 min after spinal transection), the evoked response of the dorsal horn neurons fully returned to the control (Figs. 4 and 5). In 10 of 20 rats, the inhibitory effect of intravenous morphine on the evoked response was tested again. Repeat morphine injection (2.5 mg/kg, i.v.) significantly inhibited the evoked response of 10 dorsal horn
neurons, an effect similar to that produced by first dose of morphine before spinal transection (Figs. 4 and 5).

In another 10 rats subjected to spinal cord transection, we determined the inhibitory effect of systemic morphine following blockade of spinal muscarinic receptors. Atropine (10 µM) was topically applied to the recording site of the spinal cord 30 min after spinal transection. In the presence of atropine, the inhibitory effect of the second dose of morphine (2.5 mg/kg) on the evoked response of 10 dorsal horn projection neurons was also significantly attenuated (Fig. 6). The attenuated inhibitory effect of systemic morphine on dorsal horn neurons by spinal application atropine was not significantly different from that observed in intact rats (Figs. 3 and 6).
DISCUSSION

In this study, we investigated if the inhibitory effect of systemic morphine on dorsal horn neurons is dependent upon the descending pathways. Furthermore, we determined the potential role of supraspinal descending pathways in activation of the spinal cholinergic system, which has been proposed to contribute to some of the inhibitory effect of systemic morphine on dorsal horn neurons (Chiang and Zhuo, 1989; Xu et al., 1997; Chen and Pan, 2001). Spinal dorsal horn neurons are subject to modulation by the supraspinal descending pathways. For example, stimulation of the periaqueductal gray and rostral ventromedial medulla inhibit dorsal horn neurons (Dostrovsky et al., 1983; Basbaum and Fields, 1984). However, the role of the descending pathways in the analgesic effect and inhibition of ascending dorsal horn neurons produced by systemic opioids is not fully known. The µ opioid receptors are located in various brain regions and the superficial spinal dorsal horn. Activation of µ opioid receptors in the spinal dorsal horn produces analgesia through inhibition of glutamatergic synaptic inputs and hyperpolarization of dorsal horn neurons (Schneider et al., 1998; Kohno et al., 1999). The interneurons in the dorsal horn can be either inhibitory or excitatory (Cervero and Iggo, 1980). Because opioids and muscarinic receptor agonists can excite interneurons in the dorsal horn (Sastry and Goh, 1983; Magnuson and Dickenson, 1991; Li et al., 2002; Zhang et al., 2005), we focussed our study on the spinal dorsal horn projection neurons. Intravenous morphine, at a dose producing evident analgesia in conscious rats (Chen and Pan, 2001), produced a profound inhibitory effect on all the dorsal horn projection neurons tested. Notably, only some dorsal horn projection neurons displayed increased firing activity following spinal cord transection. Since the
spinal cord was transected 30 min after the initial systemic morphine, the lack of a general increase in the dorsal horn neuronal activity after cervical spinal transection could be due to the residual morphine effect. Also, in addition to the descending inhibitory modulation, the supraspinal descending facilitatory pathway has been well recognized (Soja and Sinclair, 1983b; Porreca et al., 2001). Hence, surgical spinal cord transection may have removed the descending inhibitory as well as facilitatory influences on the dorsal horn projection neurons, resulting in lack of a net increase in the firing activity of some cells.

In the present study, we observed that the inhibitory effect of systemic morphine on dorsal horn projection neurons was not reduced by transection of the spinal cord, because morphine produced a same degree of inhibition on dorsal horn projecting neurons in both intact and spinally transected rats. Furthermore, we found that spinal application of CTAP, a specific µ opioid receptor antagonist (Kohno et al., 1999), completely blocked the inhibitory effect of systemic morphine on dorsal horn neurons. This functional evidence strongly suggests that the inhibitory effect of systemic morphine on dorsal horn neurons is mediated by direct activation of spinal µ opioid receptors. Consistent with our finding, systemic morphine is capable of suppressing the evoked responses of dorsal horn neurons even after cold block of the spinal cord in cats (Soja and Sinclair, 1983a). Also, it has been shown that morphine is more potent in increasing the nociceptive threshold when the rat spinal cord conduction is blocked (Sinclair et al., 1988). In fact, when morphine is microinjected into the periaquaductal gray, it increases the firing activity of most dorsal horn neurons (Dickenson and Le Bars, 1987). Collectively, data from this and previous electrophysiological studies suggest that the supraspinal descending inhibitory pathway is not involved in the inhibitory effect of systemic morphine on dorsal horn neurons.
The spinal cord cholinergic system plays an important role in regulation of nociception. For instance, intrathecal administration of muscarinic receptor agonists or acetylcholinesterase inhibitors produce antinociception in both animals and humans (Naguib and Yaksh, 1994; Hood et al., 1997; Naguib and Yaksh, 1997). While muscarinic receptor agonists stimulate dorsal horn inhibitory interneurons (Li et al., 2002; Zhang et al., 2005), they consistently inhibit spinal dorsal horn projection neurons (Chen and Pan, 2004). Furthermore, the spinal cholinergic system and muscarinic receptors are involved in the analgesic effect of systemic morphine in rats (Chiang and Zhuo, 1989; Chen and Pan, 2001). This is because intravenous morphine increases acetylcholine in the dialysate of the sheep spinal cord (Xu et al., 1997), and intrathecal atropine largely attenuates the analgesic effect of systemic morphine in conscious rats (Chen and Pan, 2001). Nevertheless, there is no direct functional evidence showing that the descending pathways are involved in activation of spinal cholinergic system that contribute to the inhibitory effect of systemic morphine on spinal dorsal horn neurons. Importantly, we found that spinal topical application of the muscarinic receptor antagonist atropine largely attenuated the inhibitory effect of systemic morphine on dorsal horn projection neurons, suggesting that the spinal acetylcholine muscarinic receptor is involved in the inhibitory effect of systemic morphine. In contrast to our initial hypothesis, we observed that attenuation of the inhibitory effect of systemic morphine on dorsal horn neurons by spinally applied atropine was not reduced in rats subjected to spinal cord transection. Therefore, our study provides important functional evidence that activation of the spinal μ opioid receptors stimulates the spinal cholinergic system, which in turn inhibits dorsal horn projection neurons independent of the descending pathways.

It has been suggested that the descending pathway is involved in analgesia produced by
systemic morphine (Chiang and Zhuo, 1989). Unlike our study on spinal dorsal horn neurons, the inhibitory effect of systemic morphine in that study is assessed using a withdrawal reflex test to noxious heat in lightly anesthetized rats (Chiang and Zhuo, 1989). It should be noted that because the spinal transection increases the spinal reflex activity, interpretation of the evoked reflex response is difficult in spinally transected animals (Chiang and Zhuo, 1989). Contrary to the observation that the cervical spinal cholinergic neurons originate from the brainstem in rats (Jones et al., 1986), many studies have shown that the cholinergic innervation of the spinal cord is intrinsic. In this regard, neurons and nerve terminals expressing choline acetyltransferase/acetylcholinesterase and muscarinic receptors are present in the spinal dorsal horn (Barber et al., 1984; Borges and Iversen, 1986; Wets and Vaughn, 1994; Hoglund and Baghdoyan, 1997). Furthermore, spinal cord transection does not reduce the amount of choline acetyltransferase in cats (Kanazawa et al., 1979), and retrograde axonal tracing and choline acetyltransferase immunocytochemistry reveals that none of the brainstem neurons that project to the spinal cord is cholinergic in rats (Sherriff et al., 1991). Although our data suggest that the descending pathways are less likely involved in activation of the spinal cholinergic system that mediates the inhibitory effect of systemic morphine on dorsal horn neurons, it should be acknowledged the present study was performed in anesthetized rats using a single dose of morphine. As a result, our data do not exclude the possibility that the supraspinal sites are important for the analgesic effect of systemic opioids in conscious animals or humans. In this regard, the analgesic effect produced by intracerebroventricular morphine is reversed by intrathecal adrenergic and serotoninergic receptor antagonists (Suh et al., 1989), and the same occurs when morphine is injected into the periaqueductal gray (but not the brainstem) (Fields and
In summary, this study provides new functional evidence that systemic morphine inhibits dorsal horn projection neurons through direct activation of spinal μ opioid receptors. Our data suggest that inhibition of spinal dorsal horn neurons by activation of the local spinal cholinergic circuitry following systemic morphine is independent of supraspinal descending pathways. This new information is important for our understanding of the role of spinal μ opioid receptors and cholinergic system in the analgesic action of systemic opioids.
REFERENCES


Chen SR and Pan HL (2004) Activation of muscarinic receptors inhibits spinal dorsal horn


Hoglund AU and Baghdoyan HA (1997) M2, M3 and M4, but not M1, muscarinic receptor subtypes are present in rat spinal cord. *J Pharmacol Exp Ther* **281**:470-477.


Soja PJ and Sinclair JG (1983b) Tonic descending influences on cat spinal cord dorsal horn


Footnotes

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FIGURE LEGENDS

Fig. 1. Inhibitory effect of repeat systemic morphine (2.5 mg/kg, i.v.) on the evoked response of dorsal horn projection neurons (n = 9) to pressure and pinch applied to the receptive field. The inhibitory effect of the first (M1) and second (M2) dose of morphine was measured 10 min after drug injection. Data presented as means ± SEM. * P < 0.05 compared with corresponding values in the control and recovery.

Fig. 2. Spinal topical application of 1 µM CATP completely blocked the inhibitory effect of morphine (2.5 mg/kg, i.v.) on 6 dorsal horn projection neurons. The inhibitory effect of the first (M1) and second (M2) dose of morphine was measured 10 min after drug injection. CTAP was topically applied to the lumbar spinal cord 5 min before the second dose of morphine was injected. Data presented as means ± SEM. * P < 0.05 compared with the respective control.

Fig. 3. Inhibitory effect of morphine (2.5 mg/kg, i.v.) on 11 dorsal horn projection neurons before and after spinal topical application of 10 µM of atropine. The inhibitory effect of the first (M1) and second (M2) dose of morphine was measured 10 min after each drug injection. Atropine was topically applied to the lumbar spinal cord 5 min before the second dose of morphine was injected. Data presented as means ± SEM. * P < 0.05 compared with corresponding values in the control and recovery. # P < 0.05 compared to the inhibitory effect of initial morphine.

Fig. 4. Original neurograms showing the inhibitory effect of systemic morphine (2.5 mg/kg, i.v.)
on the evoked response of an ascending dorsal horn neuron to pressure and pinch before and 1 hr after transection of the cervical spinal cord. Arrows indicate the time point of application of the stimuli to the receptive field.

Fig. 5. Inhibitory effect of morphine (2.5 mg/kg, i.v.) on 10 dorsal horn projection neurons before and after spinal cord transection. The inhibitory effect of the first (M1) and second (M2) dose of morphine was measured 10 min after each drug injection. Surgical transection of the cervical spinal cord (indicated by the arrow) was performed 30 min after the first dose of morphine was injected. Data presented as means ± SEM. * P < 0.05 compared with corresponding values in the control and recovery.

Fig. 6. Topical spinal application of atropine attenuated the inhibitory effect of systemic morphine (2.5 mg/kg, i.v.) on 10 dorsal horn projection neurons in rats subjected to spinal cord transection. The inhibitory effect of the first (M1) and second (M2) dose of morphine was measured 10 min after each drug injection. Surgical transection of the cervical spinal cord (indicated by the arrow) was performed 30 min after the first dose of morphine was injected. Atropine (10 µM) was topically applied to the lumbar spinal cord 5 min before the second dose of morphine was injected. Data presented as means ± SEM. * P < 0.05 compared with corresponding values in the control and recovery. # P < 0.05 compared to the inhibitory effect of initial morphine.
Fig. 2

- **Baseline**
- **Press**
- **Pinch**

Firing activity (Hz)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>Press</th>
<th>Pinch</th>
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<tbody>
<tr>
<td>Control</td>
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<td>M1</td>
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<td>Recovery</td>
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<td>CTAP + M2</td>
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*Significant difference from control.*
Fig. 3

Firing activity (Hz)

- **baseline**
- **press**
- **pinch**

- control
- M1
- recovery
- atropine
- atropine+M2

* and # symbols indicate significant differences.
Fig. 4
Fig. 6

The figure shows a bar graph depicting firing activity (Hz) across different conditions: control, M1, recovery, atropine, and atropine+M2. The conditions are differentiated by bars with different patterns:

- **Baseline** represented by white bars.
- **Pinch** represented by diagonal striped bars.
- **Press** represented by black bars.

Significant differences are indicated by asterisks (*) and hash symbols (#).