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Short communication

Morphine-induced decrease in mechanical allodynia is mediated by central, but not peripheral, opioid receptors in rats with inflammation

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Abstract

The aim of this study was to investigate the mechanism underlying the effect of morphine on allodynia to complete Freund's adjuvant-induced inflammation in rats. Morphine (5 mg/kg, i.v.) markedly inhibited the mechanical stimulation-induced nociceptive reflex of the gastrocnemius muscle in the inflamed hind-limb, and the inhibition was blocked by naloxone (1 mg/kg). Teased fiber recordings were made from the tibial nerve innervating the inflamed hindpaw. Morphine at the same dose did not affect the spontaneous firing rate of A-type fibers, whereas it markedly decreased the spontaneous firing of C-type fibers. The present data suggested that the central, but not peripheral, plasticity triggered by inflammation-induced facilitation of A_β fibers plays an important role in morphine-induced alleviation of allodynia, whereas activation of opioid receptor expression on the peripheral terminals of C fibers may contribute to morphine-induced alleviation of persistent pain of inflammation.

Keywords: Morphine; Inflammation; Allodynia; A-type afferent

1. Introduction

Aside from hyperalgesia and spontaneous pain, allodynia is a common and prominent symptom of inflammatory pain. The mechanism underlying allodynia is not fully clear. A line of evidence supports the hypothesis that activity and plasticity of A_β fibers are critical for triggering and maintaining allodynia (Woolf and Doubell, 1994; Neumann et al., 1996; Wasner et al., 1998). In our previous studies, after carrageenan-induced inflammation, most of the A_β neurons in the dorsal root ganglion displayed a significant increase in spontaneously ongoing activity that was rarely observed in naïve animals (Zhang et al., 2001; Xu and Zhao, 2001).

Accumulating evidence showed that morphine was effective to relieve inflammatory pain (Dickenson and Sullivan, 1986; Przewlocki and Pezewlocka, 2001) and μ-opioid receptors were identified on both peripheral and central C-fibers, but not on A_β fibers, of the axon terminals of the dorsal root ganglion neurons in the rat, particularly following peripheral inflammation (Stein et al., 1990; Coggeshall and Carlton, 1997). However, it is not know whether A_β fibers are involved in the morphine-induced decrease in allodynia. The present work was undertaken to investigate the effect of morphine on the A fiber-mediated response to mechanical stimulation as well as spontaneously ongoing firing in A- and C-type fibers following inflammation.

2. Material and methods

Experiments were carried out in 30 male Wistar rats (180–250 g). All experiments were conducted in accordance with the guidelines of the Association for the Study of Pain (IASP) concerning the use of the laboratory animals. Under ether anesthesia, complete Freund's adjuvant (300 μl, Sigma) suspended in an oil/saline (1:1) emulsion was injected at a concentration of 0.5 mg/ml into the plantar surface of the left hindpaw. Electrophysiological recordings were performed 3–6 days after the complete Freund's adjuvant injection, when significant inflammation was present in the ipsilateral hindpaw.

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Electromyography (EMG) recordings from the gastrocnemius muscles were performed as assessment of the nociceptive reflex. Rats were anesthetized with pentobarbital sodium (initially 40 mg/kg i.p.) and fixed in a stereotaxic frame. The muscle was activated by pressuring the inflamed hindpaw using Von Frey hair at a weight of 6.8 g. Such stimulus did not produce any response in the normal animal and also did not evoke any pain sensation in humans. A stable baseline was established for at least 20 min prior to drug application. The drug effect was expressed as percent change compared to baseline.

For single-unit recordings, rats were anaesthetized with pentobarbital sodium (initially 40 mg/kg i.p. and supplemented with 20 mg/kg if necessary). The trachea and jugular vein were cannulated. Core temperature, respiration, heart rate, electrocardiogram and blood pressure were continuously monitored. The left tibial nerve was exposed. As described previously (Chen et al., 1999), the tibial nerve was isolated and the perineurium was opened. Microfilaments containing one or two units discharging spontaneously were teased from the tibial nerve using forceps. The microfilaments were placed on a single Ag/AgCl recording electrode, referenced to a nearby indifferent electrode. The categorization of a single unit was determined by the conduction velocity and other established criteria of sensory properties (Lawson et al., 1996). Receptive fields of the afferents were determined by brushing, light pressure, bending of the joint or noxious stimulation. In some experiments, electrophysiological recordings were made in artificially respired paralyzed rats with Flaxedil (10 mg/kg, i.v.). Responses to drugs were significant if there was a change of at least 30% in the firing rate in 100-s duration.

All drugs were injected intravenously followed by 0.2-ml saline. Administration of 5 mg/kg morphine was completed within 5 s. Naloxone (1 mg/kg) was given 3 min before the application of morphine.

Data are presented as means ± S.D. and were subjected to statistical evaluation using two-tailed Student's *t* test. Criteria for significance in all analyses were defined as $P < 0.05$.

3. Results

3.1. Effect of morphine on mechanical allodynia

Three to six days after injection of complete Freund's Adjuvant, an EMG recording from the gastrocnemius muscle was evoked by von Frey hair pressuring (6.8 g) to the inflamed hindpaw. In the contralateral gastrocnemius muscle (non-inflamed side), the same intensity of mechanical stimulation failed to evoke an EMG recording. Therefore, an EMG recording from the inflamed hindlimb was characterized as a nociceptive reflex, which is a marker for allodynia. Intravenous injection of morphine (5 mg/kg) greatly depressed the mechanical stimulation-induced EMG. The greatest inhibition by morphine was 90.35 ± 6.9% ($P < 0.01$) (Fig. 1). Morphine-induced inhibition was blocked by pre-administration of naloxone (1 mg/kg).

3.2. No effect of morphine on the spontaneous discharge in A-type fibers

Single fiber recording was made from the tibial nerve innervating the inflamed hindpaw. Thirty spontaneously discharging mechanosensitive afferents were recorded. The mean conduction velocity and spike duration were 35.45 ± 10.40 m/s and 0.61 ± 0.07 ms, respectively. Half of the units (50.15%) had a regular interspike interval ranging from 17 to 50 ms (38.26 ± 10.81 ms). These fibers, probably originating from muscles or joints, were often sensitive to pressure or bending of the hindpaw. Some fibers with irregular interspike intervals and discharging at lower rate were often sensitive to brushing or touching of the skin. Based on the conduction velocity, mechanosensitivity, spike size and duration, all the recorded units were characterized as A-type fibers and most were A_β fibers.

No detectable effect of morphine (5 mg/kg, i.v.) was observed on the spontaneously ongoing discharges in 29 out of 30 A-type fibers (Fig. 2A) except for one neuron, of which discharges were reduced by less than 10%. Also, morphine failed to affect the mechanical stimulation-induced responses in the three A-type fibers touching a receptive field of skin in the hindpaw.

Given that inflammation produces an increase in spontaneously ongoing firing of nociceptors, which is correlated with the persistent pain (Xu et al., 2000; Koltzenburg, 1999), three spontaneously discharging C fiber afferents in the tibial nerve were recorded. These fibers were sensitive to noxious stimulation of the skin (noxious pinch or heating) and the mean conduction velocity was 1.0 ± 0.4 m/s ($n = 3$). In contrast to the A fiber test, morphine at the

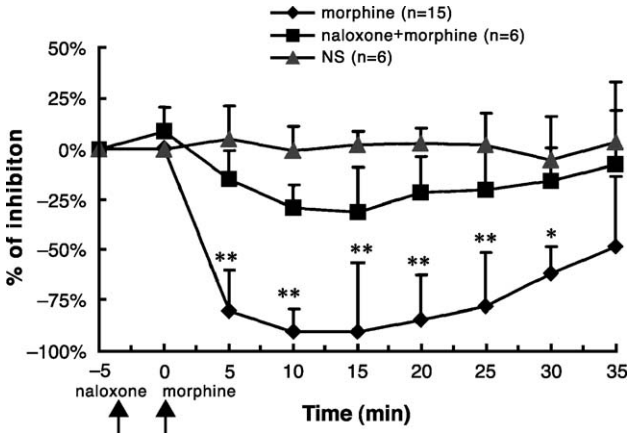

Fig. 1. Inhibitory effect of morphine (5 mg/kg, i.v.) on mechanical allodynia in the rat with inflammation. EMG recording from the gastrocnemius muscle was evoked by von Frey hair pressure (6.8 g) to the inflamed hindpaw. Morphine-induced inhibition was blocked by naloxone (1 mg/kg, i.v.). $^{**}P < 0.01$; $^{*}P < 0.05$.

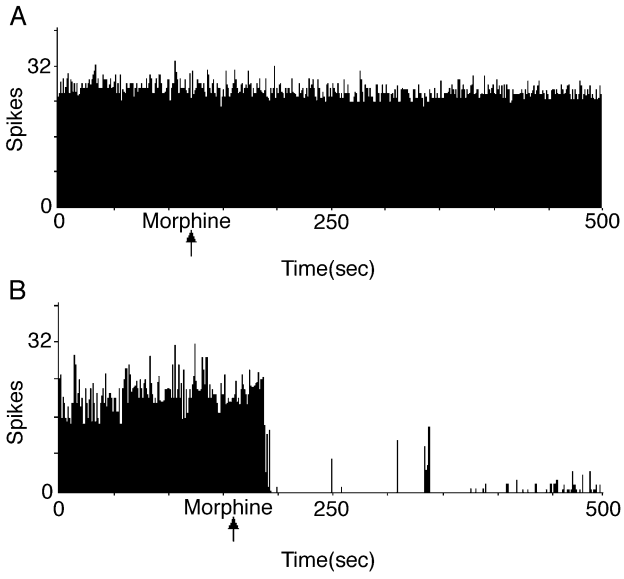

Fig. 2. Morphine (5 mg/kg, i.v.) inhibited the spontaneous discharges in single C-type fiber (B), but not in single A-type fiber (A), recorded from the inflamed hindpaw.

same dose almost completely depressed the spontaneous discharge of all three C fibers tested (Fig. 2B). The depression did not recover within 15 min.

4. Discussion

In the present study, EMG recorded from the gastrocnemius muscle was evoked by Von Frey hair pressuring to the inflamed hindpaw, but such innocuous stimuli failed to evoke EMG recordings in the contralateral gastrocnemius muscle (non-inflamed side). Therefore, it could be considered that as EMG recording in the inflamed hindlimb characterizes a nociceptive reflex, which is a marker for allodynia. The present results showed that systematic administration of morphine significantly reduced mechanical stimulation-induced allodynia, whereas it failed to affect spontaneous discharges in A-type fibers of the tibial nerve innervating the inflamed hindpaw. Some studies have demonstrated that spontaneous firing from A_β fibers increased significantly in rats with inflammation. Such inflammation-induced facilitation of A_β fibers increases excitability of spinal cord neurons which contributes to generation of allodynia (Chen et al., 1999; Wasner et al., 1998; Xu and Zhao, 2001; Zhang et al., 2001). Anatomical studies showed that large-diameter neurons in the dorsal root ganglion did not contain substance P mediating nociceptive information under the physiological conditions, but these neurons synthesized de novo and released substance P following peripheral inflammation (Neumann et al., 1996; Xu et al., 2000). It has also been observed that peripheral inflammation could induce A-fiber afferents to sprout in the lamina II of the spinal dorsal horn, which leads to novel synaptic connection mediating nociceptive processing (Neumann et al., 1996; Ossipov et al., 2000). Thus, the present finding that morphine inhibited the spinal nociceptive reflex, but not spontaneous and mechanical stimulation-evoked discharges of A-type fibers, strongly suggested that a central, but not a peripheral, mechanism is involved in morphine-induced reduction of allodynia (Cahill et al., 2003).

It is consistent with earlier results (Jiang et al., 1994; Chen et al., 1995) that morphine-induced inhibition of spontaneous discharges in C fibers was observed in this study. It has been shown that μ-opioid receptors predominantly exist on both peripheral and central terminals of primary afferent C fibers, but not in A_β fibers (Coggeshall and Carlton, 1997; Stein et al., 2001; Städer et al., 2002). Following peripheral inflammation, the density of opioid receptors is up-regulated on peripheral nerve terminals (Stanfa et al., 1992; Hassan et al., 1993; Stein et al., 2001), and spontaneous ongoing firing of nociceptors, which correlates with the persistent pain, increases markedly (Reeve et al., 1998; Koltzenburg, 1999; Koltzenburg et al., 1994; Ossipov et al., 2000; Xu et al., 2000). In addition to the central mechanism (Dickenson and Sullivan, 1986; Marsh et al., 1999; Przewlocki and Pezewlocka, 2001), it is therefore conceivable that morphine binding to μ-opioid receptors on C-fiber terminals within the peripheral tissue resulted in inhibition of firing in C fibers innervating the inflamed area, further supporting the view that a peripheral mechanism is involved in the μ-opioid receptor-induced alleviation of the persistent pain (Stein et al., 1990, 2001; Jin et al., 1999).

In conclusion, central, but not peripheral, plasticity triggered by inflammation-induced facilitation of A_β fibers play an important role in the morphine-induced alleviation of mechanical allodynia, whereas activation of μ-opioid receptors on the peripheral terminals of C fibers contributes to the morphine-induced alleviation of hyperalgesia in inflammation.

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