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Long-term synaptic plasticity in the spinal dorsal horn and its modulation by electroacupuncture in rats with neuropathic pain

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Abstract

Our previous study has reported that electroacupuncture (EA) at low frequency of 2 Hz had greater and more prolonged analgesic effects on mechanical allodynia and thermal hyperalgesia than that EA at high frequency of 100 Hz in rats with neuropathic pain. However, how EA at different frequencies produces distinct analgesic effects on neuropathic pain is unclear. Neuronal plastic changes in spinal cord might contribute to the development and maintenance of neuropathic pain. In the present study, we investigated changes of spinal synaptic plasticity in the development of neuropathic pain and its modulation by EA in rats with neuropathic pain. Field potentials of spinal dorsal horn neurons were recorded extracellularly in sham-operated rats and in rats with spinal nerve ligation (SNL). We found for the first time that the threshold for inducing long-term potentiation (LTP) of C-fiber-evoked potentials in dorsal horn was significantly lower in SNL rats than that in sham-operated rats. The threshold for evoking the C-fiber-evoked field potentials was also significantly lower, and the amplitude of the field potentials was higher in SNL rats as compared with those in the control rats. EA at low frequency of 2 Hz applied on acupoints ST 36 and SP 6, which was effective in treatment of neuropathic pain, induced long-term depression (LTD) of the C-fiber-evoked potentials in SNL rats. This effect could be blocked by *N*-methyl-D-aspartic acid (NMDA) receptor antagonist MK-801 and by opioid receptor antagonist naloxone. In contrast, EA at high frequency of 100 Hz, which was not effective in treatment of neuropathic pain, induced LTP in SNL rats but LTD in sham-operated rats. Unlike the 2 Hz EA-induced LTD in SNL rats, the 100 Hz EA-induced LTD in sham-operated rats was dependent on the endogenous GABAergic and serotonergic inhibitory system. Results from our present study suggest that (1) hyperexcitability in the spinal nociceptive synaptic transmission may occur after nerve injury, which may contribute to the development of neuropathic pain; (2) EA at low or high frequency has a different effect on modulating spinal synaptic plasticities in rats with neuropathic pain. The different modulation on spinal LTD or LTP by low- or high-frequency EA may be a potential mechanism of different analgesic effects of EA on neuropathic pain. LTD of synaptic strength in the spinal dorsal horn in SNL rats may contribute to the long-lasting analgesic effects of EA at 2 Hz.

Keywords: Neuropathic pain; Long-term potentiation; Long-term depression; Opioid; NMDA receptor; Electroacupuncture; Spinal dorsal horn; Electrophysiology

Introduction

The underlying spinal mechanisms for the development and maintenance of neuropathic pain characterized by spontaneous pain, hyperalgesia, and allodynia (Bridges et al., 2001; Cavenagh et al., 2006) are still not fully understood. Early studies indicate that the increased sensitivity to noxious stimuli and evoked pain following innocuous stimuli could result from either a reduction in the thresholds of cutaneous nociceptors (peripheral sensitization) or an increase in the excitability of the central nervous system (central sensitization) (Woolf, 1983; Dubner, 1991; Zimmermann, 2001; Bridges et al., 2001). It is proposed that plastic changes in spinal nociceptive synapses after nerve injury may contribute to the development of central sensitization in the spinal dorsal horn and ultimately lead to the generation of neuropathic pain (Miletic and Miletic, 2000; Draganic et al., 2001). Long-term potentiation (LTP) and long-term depression (LTD), which are considered electrophysiological correlates of plastic, long-lasting changes in the efficacy of spinal synaptic transmission (Randic, 1996; Sandkuhler, 2000), may occur following peripheral nerve injury. In normal rats,

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electrical and natural noxious stimuli of afferent C-fibers or acute nerve injury could produce LTP of C-fiber-evoked field potentials in the spinal dorsal horn (Liu and Sandkuhler, 1995, 1997; Sandkuhler and Liu, 1998). However, it is unknown if and how spinal LTP changes under chronic pathological pain conditions. Thus, the first aim of the present study was to investigate the roles of spinal synaptic plasticity in the development of neuropathic pain by assessing the effects of nociceptive conditioning stimulation on the induction of LTP of C-fiber-evoked potentials in rats with neuropathic pain.

It has been reported that repetitive low-frequency electrical stimulation of primary afferent Aδ-fibers in normal rats could evoke LTD of synaptic strength in the spinal dorsal horn (Liu et al., 1998). Furthermore, transcutaneous electrical nerve stimulation (TENS) and EA have been demonstrated to be effective in alleviating neuropathic pain in humans and in animal models as well (Nam et al., 2001; Hwang et al., 2002; Kim et al., 2004). Results from our laboratory also show that EA in rats decreased spinal nerve ligation (SNL)-induced neuropathic pain behaviors in a frequency-dependent manner (Sun et al., 2002; Han, 2003; Sun et al., 2004). It is found that EA at low frequency of 2 Hz had greater and more prolonged effects on mechanical allodynia and thermal hyperalgesia than that EA at high frequency of 100 Hz in rats with neuropathic pain (Sun et al., 2002, 2004; Han, 2003). However, the mechanisms underlying the analgesic effects of TENS or EA on neuropathic pain are unclear. Therefore, the second aim of the current study was to investigate the potential mechanisms of the different analgesic effects of EA at low and high frequency in neuropathic pain by examining the effects of EA at low and high frequency on the modulation of spinal synaptic plasticities like LTP or LTD. It is of great significance to study if spinal LTD is underlying the antinociceptive effects of EA on neuropathic pain. The effectiveness of EA on the modulation of spinal LTP/LTD will provide further evidence that the spinal synaptic plasticity indeed underlies the development of neuropathic pain.

Methods

Animals

Male Sprague–Dawley rats aged 8–10 weeks were provided by the Department of Experimental Animal Sciences, Peking University Health Science Center. The animals were housed in plastic cages (up to four per cage) with soft bedding under a natural diurnal cycle at room temperature. They were provided with water and food ad libitum and raised at least 7 days before surgery. The experiments were conducted in accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and were approved by the Animal Care and Use Committee of our university.

Spinal nerve ligation (SNL)

Under general anesthesia with chloral hydrate (300 mg kg^{-1}), the left L5 and L6 spinal nerves distal to the dorsal root ganglia were tightly ligated with 4-0 silk sutures as described earlier by Kim and Chung (1992). Control animals received sham surgery with identical procedure except for ligation of the L5/L6 spinal nerves. Animals were allowed to recover for 7 days before electrophysiological recordings. Any rats exhibiting motor deficiency or lack of tactile allodynia were excluded from the study.

Mechanical allodynia, as a behavioral measure of neuropathic pain, was assessed by measuring the 50% paw withdrawal threshold (PWT) as described in our previous reports (Sun et al., 2004, 2005a,b). The experimenters were kept blind from the conditions of the rats (SNL vs. sham-operation). The 50% PWT in response to a series of von Frey filaments was determined by the up and down method (Chaplan et al., 1994) and was calculated as previously described (Dixon, 1980). The 50% PWT was obtained for all animals 1–2 days before surgery and day 7 prior to electrophysiological recordings.

Electrophysiological recording

Surgery

The rat was anesthetized and maintained with urethane (1.2–1.5 g kg^{-1}, i.p.). A tracheotomy was performed to maintain an open, low-resistance airway. A cannula was inserted into the right jugular vein for continuous infusion of Tyrode's solution (in mmol l^{-1}: NaCl 137, KCl 2.7, $CaCl_2$ 1.4, $MgCl_2$ 1.0, $NaHCO_3$ 6.0, NaH_2PO_4 2.1, D-(+)-glucose 6.5; pH 7.4) at a rate of 1.0–1.5 ml h^{-1}. Body temperature was maintained at 36.5–37.5 °C via a feedback-controlled under-body heating pad. A pair of bipolar silver hook electrode was placed under the sciatic nerve immediately proximal to the trifurcation for electrical stimulation. The vertebral column was rigidly fixed in the frame with two clamps. The lumbar enlargement of the spinal cord was exposed by laminectomy at the vertebrae T13 and L1 and the dura covering lumbosacral spinal segments was carefully removed. The exposed spinal tissue was covered with warm (37 °C) paraffin oil.

Field potential recording

The C-fiber-evoked field potentials were recorded at a depth of 100–500 μm from the dorsal surface of L4–L5 spinal cord with parylene-coated tungsten microelectrodes (impedance 1–3 MΩ, FHC, USA) driven by a micro-stepping motor. A bandwidth of 0.1–300 Hz was used to remove artifacts without altering the C-fiber-evoked field potentials. The signals were amplified, filtered and displayed on an oscilloscope, and fed to a Pentium computer via a CED 1401 interface for off-line analysis using the Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

The test stimulation of a single square pulse (0.5 ms, delivered every 2 s) was applied to the sciatic nerve to measure the threshold for evoking the field potentials. The intensity of the stimulation was increased gradually from 0 V to the voltage intensity just evoking the C-fiber-evoked field potentials as described by Liu and Sandkuhler (1998). The intensity of the stimulation that would just evoke the C-fiber-evoked field potentials was defined as the threshold for evoking the field potentials. Following this measurement, another test stimulation of a single square pulse (20 V, 0.5 ms, delivered every 2 s) was applied to measure the amplitude of the field potentials.

Induction of LTP of the C-fiber-evoked field potentials

LTP of the C-fiber-evoked field potentials was induced as described by Liu et al. (Liu and Sandkuhler, 1998). Briefly, the test stimulation of a single square pulse (10–20 V, 0.5 ms, delivered every 5 min) was applied to the sciatic nerve to evoke spinal field potentials for at least 30 min as baseline control. The mean amplitude of the control potentials was obtained from an average of 6 individual test potentials (100%). The conditioning stimulation of either high-frequency, low-intensity (HF-LI) (10 V, 0.5 ms, 100 Hz, 400 pulses given in 4 trains of 1 s duration at 10 s intervals), or high-frequency, high-intensity (HF-HI) (30–40 V, other parameters were kept as the same) was then delivered to the sciatic nerve. After the conditioning stimulation, the same test stimulation was delivered again to the sciatic nerve and the recording was continued for another 2–3 h. The amplitude of the field potential following each conditioning stimulation was normalized and expressed as the percentage of the control value.

Electroacupuncture (EA) application

In these experiments, the field potentials evoked by the test stimulation were kept stable for 60 min as control, which was averaged from 12 individual test potentials. EA stimulation at C-fiber strength was applied to the "acupoints" in the leg for the induction of LTD or LTP in the spinal dorsal horn. The procedure was similar to that used in our previous reports (Wan et al., 2001; Huang et al., 2004; Sun et al., 2004). Briefly, a pair of stainless-steel needle (0.4 mm in diameter, 5 mm in length) was inserted into acupoints "Zusanli" (ST 36, 4 mm lateral to the anterior tubercle of the tibia, which is marked by a notch) and "Sanyinjiao" (SP 6, 3 mm proximal to the medial melleolus, at the posterior border of the tibia). The electrical stimulation generated from the Han's Acupoint Nerve Stimulator (HANS, LH series, Peking University, Beijing, China) was delivered to both hind limbs simultaneously. These electric stimuli were set as square waves, 0.2 ms in duration and 100 Hz in frequency, or 0.6 ms in duration and 2 Hz in frequency. The electrical pulses were delivered from 1 to 3 mA in a step of 1 mA increment. Each step of stimulus lasted for 10 min. After EA stimulation, the test stimulation was again delivered to the sciatic nerve and the recording was continued for another 2–3 h. The amplitude of the field potential following EA stimulation was normalized and expressed as the percentage of the control value.

At the end of each experiment, the recording site was marked by electrolytic lesion (20.0 μA positive depolarizing DC current for 20 s). The animal was then euthanized by an overdose of pentobarbital sodium. The spinal cord was sectioned into 20-μm-thick transverse sections on a cryostat and stained with cresyl violet. Recording site was identified and plotted on a schematic representation of the lumbar spinal cord.

Chemical application

All chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The stock solution was dissolved in normal saline and diluted to the desired concentration in Tyrode's solution on the day of recording. All agents were administrated intravenously (i.v.) at a concentration of 1.0 mg ml^{-1}. The doses of the antagonists were determined based on our preliminary observation and reports from other laboratories. Briefly, NMDA receptor antagonist MK-801 was given at 0.5 mg kg^{-1} according to Liu and Sandkuhler (1998); opioid receptor antagonist naloxone was applied at 1.0 mg kg^{-1} according to Ulugol et al. (2002) and our previous reports (Sun et al., 2004); GABA-A receptor antagonist bicuculline was administrated at 1.0 mg kg^{-1} according to Miletic and Miletic (2001), and non-selective 5-HT receptor antagonist methysergide was applied at 5.0 mg kg^{-1} following the report of Terenzi and Prado (1990). All agents were applied about 1 min before EA application.

Statistical analysis

All data are expressed as mean±S.E.M. Student's *t* test was used for the comparison between the SNL and the sham-operated groups, and repeated measures of ANOVA followed by Newman–Keul's post-hoc test was used for the comparison between the pre- and post-conditioning stimulation (for LTP induction) or between the pre- and post-EA application (for observation on the EA effect). Statistical significance was determined as $P<0.05$.

Results

Mechanical allodynia in SNL rats

Mechanical allodynia, as a behavioral measure of neuropathic pain, was assessed by measuring the 50% paw withdrawal threshold (PWT). In 43 SNL rats, the 50% PWT was reduced significantly from 14.2±0.5 g prior to surgery to 1.6±0.3 g ($P<0.01$) at day 7 post-SNL, indicating the development of mechanical allodynia, a behavior sign of neuropathic pain. As expected, there was no significant mechanical allodynia in the 30 sham-operated rats.

High-frequency, low-intensity conditioning stimulation induced prolonged LTP in SNL rats but not in the sham-operated rats

To examine whether spinal synaptic plasticity was altered under neuropathic pain conditions, the induction of LTP of the C-fiber-evoked potentials in the spinal dorsal horn was tested in SNL and in sham-operated rats. In SNL rats, high-frequency, low-intensity (HF-LI) conditioning stimulation of the sciatic nerve induced prolonged increase (177.4±1.6% of the baseline mean control value, $P<0.001$, $n=6$, Fig. 1A) in the amplitudes of the C-fiber-evoked potentials. This enhancement lasted for more than 3 h until experiment termination. This suggested that, after peripheral nerve injury, HF-LI stimulation of the sciatic nerve could induce LTP of the C-fiber-evoked potentials in the spinal dorsal horn. However, in sham-operated rats, the same HF-LI stimulation could not induce any LTP of the C-fiber-evoked potentials. The amplitude of the C-fiber-evoked potentials was 98.6±2.9% of the baseline mean control values after HF-LI stimulation of the sciatic nerve in sham-operated rats ($P>0.05$, $n=5$, Fig. 1B). In the same group of sham-operated rats, only high-frequency, high-intensity (HF-HI) conditioning

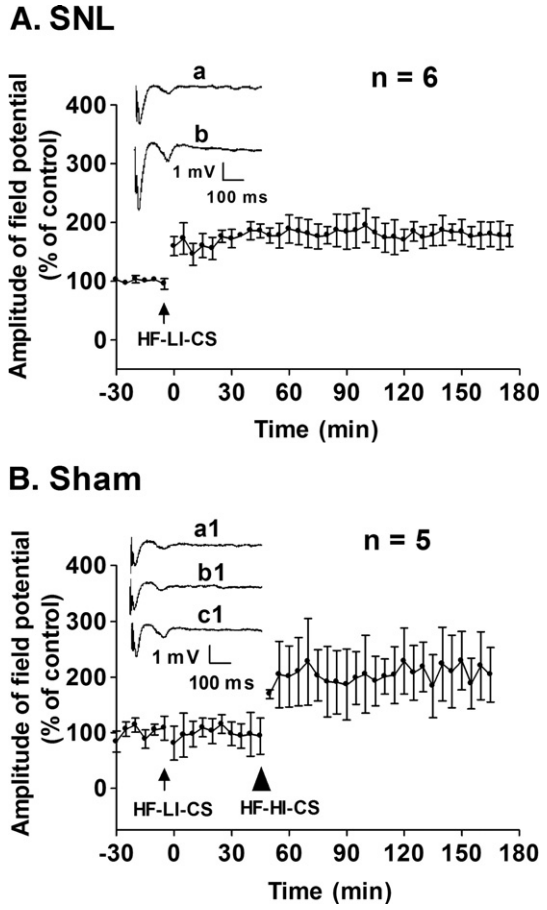

Fig. 1. Induction of LTP of the C-fiber-evoked potentials in SNL rats and in sham-operated rats. (A) In SNL rats. Six test stimuli (10–20 V, 0.5 ms, delivered every 5 min) were applied to sciatic afferents during a 30-min pre-tetanic period. Then, an HF-LI-CS (10 V, 0.5 ms, 100 Hz, 400 pulses given in 4 trains of 1 s duration at 10 s intervals) was delivered at the time point as arrowhead shows. Post-tetanic responses were recorded every 5 min for up to 3 h post-tetanus. Note that the HF-LI-CS produced a long-lasting potentiation in the evoked activities. Two individual potentials recorded in an animal are shown in inlets. Trace in (a) is an example of a recording before HF-LI-CS, trace in (b) was recorded after HF-LI-CS. (B) In sham-operated rats. The same HF-LI-CS did not produce any significant potentiation in the evoked activities. After 50 min, an HF-HI-CS (30–40 V, other parameters are the same as in HF-LI-CS) was delivered. It produced a significant LTP. Trace in (a1) is an example of a recording before HF-LI-CS. Trace in (b1) and trace in (c1) were recorded after HF-LI-CS and HF-HI-CS, respectively. HF-LI-CS: high-frequency, low-intensity conditioning stimulation. HF-HI-CS: high-frequency, high-intensity conditioning stimulation.

stimulation induced LTP (204.0±3.1% of the control value, $P<0.001$, $n=5$, Fig. 1B).

Lowered activating threshold and increased amplitude of the C-fiber-evoked field potentials in SNL rats

To further investigate if the hyperexcitability in the spinal nociceptive synaptic transmission occurred after nerve injury, the threshold for evoking the C-fiber-evoked field potentials and the amplitude of the field potentials evoked by test stimulation of a single square pulse (20 V, 0.5 ms, delivered every 5 min) were also examined in SNL and in sham-operated rats. In SNL rats, the threshold for evoking the field potentials was 6.1±0.2 V in response to the test stimulation, which was significantly lower than that (13.3±0.4 V) in sham-operated rats ($P<0.001$, Fig. 2A). Moreover, the amplitude of the field potentials evoked by test stimulation was also significantly increased (0.8±0.1 mV) in SNL rats as compared to that (0.4±0.4 mV) in sham-operated rats ($P<0.001$, Fig. 2B). These results suggested that hyperexcitability in the spinal nociceptive synaptic transmission may occur after nerve injury.

Low-frequency (2 Hz) EA induced spinal LTD in SNL rats

EA at low frequency could alleviate neuropathic pain (Sun et al., 2002, 2004; Han, 2003). To explore the potential mechanism of its action, we examined the effect of EA at low frequency of 2 Hz on spinal synaptic plasticity in neuropathic pain. In SNL rats, EA at 2 Hz to acupoints ST 36 and SP 6 for 30 min decreased the amplitudes of the field potentials to 49.4±0.6% of the control value ($P<0.001$, $n=6$, Fig. 3A). The depression lasted for more than 3 h until experiment termination, which suggests that 2 Hz EA could induce LTD in the spinal dorsal horn. As expected, the sham EA (needling without electrical stimulation) failed to induce any LTD in the spinal dorsal horn (Fig. 3B).

Low-frequency (2 Hz) EA-induced LTD was blocked by MK-801 or naloxone but not by bicuculline or methysergide

Then, we investigated the possible mechanisms underlying the 2 Hz EA-induced LTD in SNL rats. We found that the 2 Hz EA-induced LTD could be blocked by pretreatment with the NMDA receptor antagonist MK-801 or the opioid receptor antagonist naloxone, but not by the GABA-A receptor antagonist

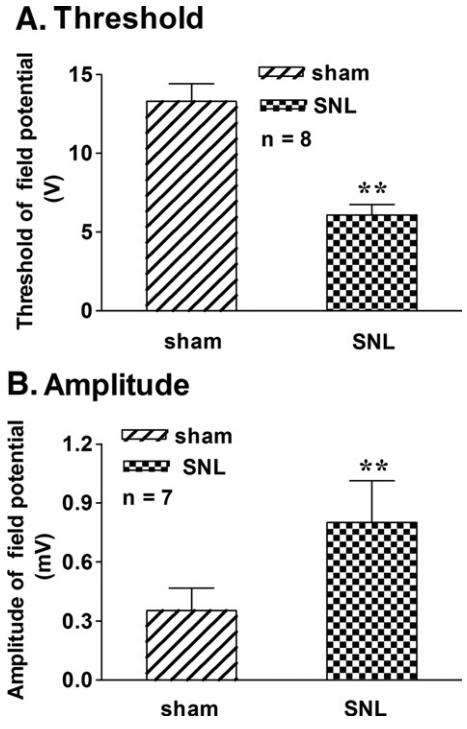

Fig. 2. Threshold and amplitude of the C-fiber-evoked field potentials in SNL rats and in the sham-operated rats. (A) Threshold. (B) Amplitude. **$P<0.001$ as compared with the sham-operated group.

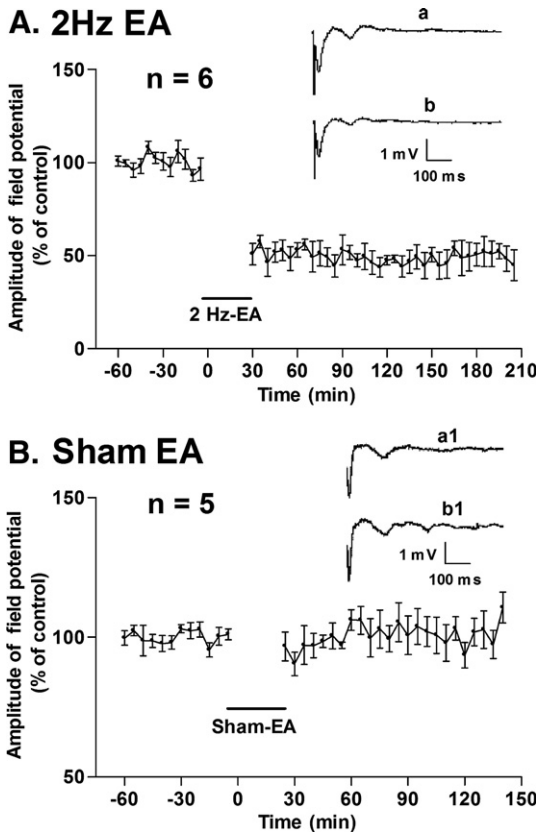

Fig. 3. 2 Hz EA induced LTD of the C-fiber-evoked potentials in the spinal dorsal horn in SNL rats. (A) 2 Hz EA. A significant LTD was induced after EA application. In SNL rats, 12 test stimuli (10–20 V, 0.5 ms, delivered every 5 min) were applied to sciatic afferents during a 60-min period before EA. Post-EA responses were recorded every 5 min for up to 3 h. Two individual potentials recorded in an animal are shown in inlets. Trace in (a) is an example of a recording before EA, trace in (b) was recorded after EA. (B) Sham EA. No significant LTD was induced after sham EA application. Trace in (a1) is an example of a recording before sham EA, trace in (b1) was recorded after sham EA.

bicuculline or the non-selective 5-HT receptor antagonist methysergide. In SNL rats pretreated with MK-801 (0.5 mg kg^{-1}) or naloxone (1.0 mg kg^{-1}), the amplitudes of the C-fiber-evoked potentials remained at 98.8±0.8% ($P>0.05$, $n=6$, Fig. 4A) or 99.2±1.1% of the control value ($P>0.05$, $n=5$, Fig. 4B) after 2 Hz EA, suggesting that MK-801 or naloxone could block the 2 Hz EA-induced LTD. However, in SNL rats pretreated with bicuculline (1.0 mg kg^{-1}) or methysergide (5.0 mg kg^{-1}), the amplitudes of the C-fiber-evoked potentials were 56.2±0.7% ($P<0.001$, $n=5$, Fig. 4C) or 57.2±0.7% of the mean control value ($P<0.001$, $n=5$, Fig. 4D) after 2 Hz EA, indicating that bicuculline or methysergide had no effect on the 2 Hz EA-induced LTD. As a control, MK-801 or naloxone alone was tested and showed no significant effect on the C-fiber-evoked potentials (data not shown).

High-frequency (100 Hz) EA-induced LTP in SNL rats and LTD in sham-operated rats

Finally, we examined the effect of high-frequency EA (100 Hz) on spinal synaptic plasticity in neuropathic pain. Interestingly, 100 Hz EA induced LTP instead of LTD in the spinal dorsal horn in SNL rats. The amplitudes of the C-fiber-evoked potentials increased to 175.3±1.9% of the control value after EA application ($P<0.001$, $n=5$, Fig. 5A). Unexpectedly, in sham-operated rats, 100 Hz EA induced LTD rather than LTP. The amplitudes of the C-fiber-evoked potentials were reduced to 63.7±0.6% of the control value ($P<0.001$, $n=5$, Fig. 5B) after EA application.

High-frequency (100 Hz) EA-induced LTD was blocked by a combination of bicuculline with methysergide in sham-operated rats

Moreover, we investigated the possible mechanism by which 100 Hz EA induced LTP in SNL rats but LTD in sham-operated rats. In the sham-operated rats, pretreatment with naloxone (1.0 mg kg^{-1}), bicuculline (1.0 mg kg^{-1}), or methysergide (5.0 mg kg^{-1}) alone could not block the 100 Hz EA-induced LTD. The amplitudes of the C-fiber-evoked potentials were 57.6±0.9% ($P<0.001$, $n=5$, Fig. 6A), 57.1±0.6% ($P<0.001$, $n=5$, Fig. 6B), or 61.6±0.7% ($P<0.001$, $n=5$, Fig. 6C) of the mean control values, respectively, after 100 Hz EA application. However, a combined pretreatment with bicuculline (1.0 mg kg^{-1}) and methysergide (5.0 mg kg^{-1}) not only blocked the 100 Hz EA-induced LTD but also converted LTD to LTP in the sham-operated rats. The amplitude of the C-fiber-evoked potentials was 114.0±0.6% after 100 Hz EA application ($P<0.001$, $n=5$, Fig. 6D).

Discussion

Spinal LTP in the development of neuropathic pain

In the present study, we demonstrated for the first time that the threshold for evoking spinal LTP was significantly decreased in rats with spinal nerve injury. In addition, spinal nerve injury decreased the threshold of C-fiber-evoked potentials and increased the amplitude of the field potentials.

It is postulated that spinal central sensitization under chronic pain conditions results from plastic changes in the processing of sensory, particularly nociceptive information (Amantea et al., 2000; Sandkuhler, 2000; Woolf and Salter, 2000; Zimmermann, 2001; Schaible and Richter, 2004; Campbell and Meyer, 2006). LTP, an activity-dependent increase in synaptic transmission, was firstly described in hippocampus and believed to be one of the fundamental mechanisms of learning and memory (Bliss and Lomo, 1973; Bliss and Collingridge, 1993). Several studies have shown that LTP-like phenomena can be induced not only in the brain but also in the spinal cord (Liu and Sandkuhler, 1995, 1997, 1998; Sandkuhler and Liu, 1998). In normal rats, electrical or natural noxious stimulation of afferent C-fibers or acute nerve injury produces LTP of C-fiber-evoked field potentials in the spinal dorsal horn (Liu and Sandkuhler, 1997; Sandkuhler and Liu, 1998). Increased nociceptor activity following trauma may also lead to a long-term increase of the excitability of wide dynamic range (WDR) neurons in the spinal dorsal horn with a sustained depolarization and/or a gain in

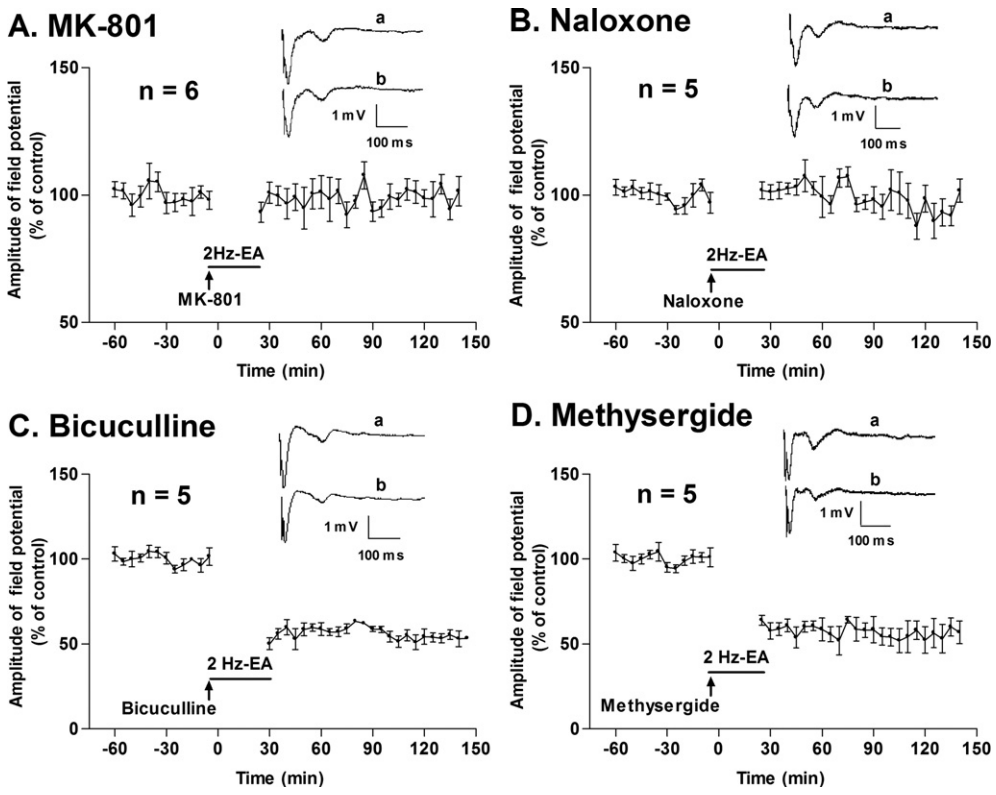

Fig. 4. 2 Hz EA-induced LTD in SNL rats was blocked by MK-801 or naloxone but not by bicuculline or methysergide. (A) MK-801, (B) naloxone, (C) bicuculline, (D) methysergide. Two individual potentials recorded in an animal are shown in inlets. Trace in (a) is an example of a recording before EA, trace in (b) was recorded after EA.

transmitter release from the afferent fibers terminating on these neurons (Svendsen et al., 1997, 1999, 2000; Rygh et al., 1999). Intense noxious stimulation of peripheral tissue or direct nerve injury produces hyperalgesia, an increased response to noxious stimulation (Dubner, 1991).

It is believed that sensitization of both peripheral nociceptors and the spinal dorsal horn neurons is responsible for the abnormal pain sensation (Woolf, 1983; Dubner, 1991; Sandkuhler, 2000; Woolf and Salter, 2000; Zimmermann, 2001; Schaible and Richter, 2004). Our findings showed that the threshold for evoking spinal LTP was significantly decreased after SNL, which suggest that LTP of the C-fiber-evoked potentials, an index of enhancement of synaptic transmission between afferent C-fibers and the neurons of the spinal dorsal horn, may underlie the central mechanism of neuropathic pain. After spinal nerve injury, the threshold for evoking the C-fiber-evoked field potentials was also significantly lower, and the amplitude of the field potentials was higher in SNL rats as compared with the control rats. These results indicate a functional increase in dorsal horn neuronal excitability, and hyperexcitability in the spinal nociceptive synaptic transmission may occur after nerve injury, which may contribute to the development of neuropathic pain.

Our previous studies and those of others have shown that ectopic discharges from the injured nerve fibers are highly correlated with tactile allodynia only in early, but not in late stage of neuropathic pain after nerve injury (Sun et al., 2005a,b; Xie et al., 2005). It is highly likely that ectopic discharges from the injury sites and the dorsal root ganglion neurons contribute to the initiation of neuropathic pain in the early stage, while, spinal LTP, which might be induced by ectopic discharges, plays more important roles in the development and maintenance of neuropathic pain in the late stage.

Significance and mechanisms of EA-induced spinal LTD

Works from our laboratory and others have shown that TENS or EA had long-lasting analgesic or antinociceptive effects in a frequency-dependent manner (Dai et al., 2001; Hwang et al., 2002; Rapson et al., 2003; Sun et al., 2002, 2004; Zhang et al., 2004; Kim et al., 2005; Somers and Clemente, 2006). Opioid receptors and NMDA receptors are believed to participate in the long-lasting analgesic effects of TENS or EA (Han, 2003, 2004; Sun et al., 2004; Kim et al., 2004; Zhang et al., 2004). In the present study, we demonstrated that EA at 2 Hz induced significant LTD of the C-fiber-evoked potentials in SNL rats. On the contrary, EA at 100 Hz induced spinal LTP in SNL rats but LTD in sham-operated rats. Our findings provide a convincing explanation for the differential antinociceptive effects of EA at low and at high frequencies on neuropathic pain through modulation of spinal synaptic plasticities, e.g., LTD or LTP. The results also provide a solid electrophysiological evidence that LTD of synaptic strength in the spinal dorsal horn may be a potential mechanism underlying the long-lasting antinociceptive effects produced by TENS or EA (Sandkuhler et al., 1997; Liu et al., 1998; Ikeda et al., 1999; Sandkuhler, 2000).

Our results further showed that the 2 Hz EA-induced spinal LTD could be blocked by NMDA receptor antagonist MK-801 or by opioid receptor antagonist naloxone, but not by GABA-A

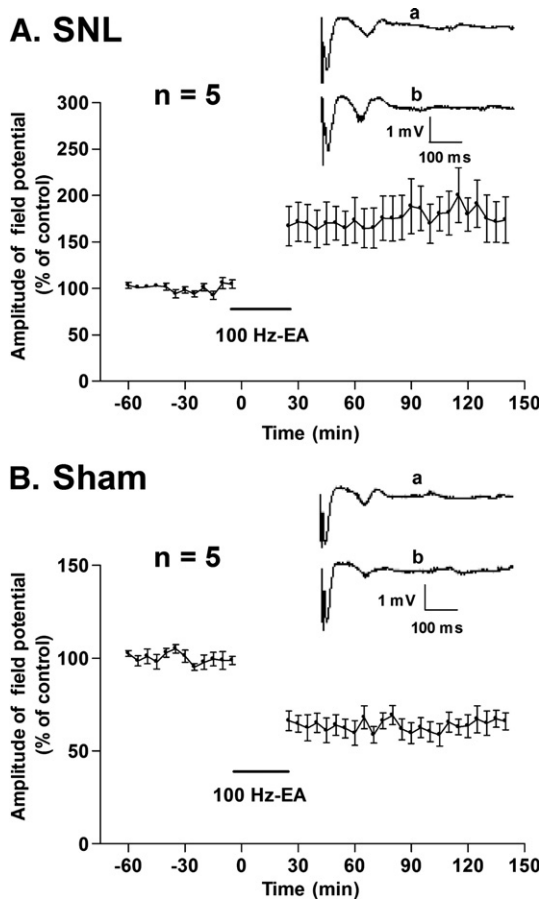

Fig. 5. 100 Hz EA induced LTP of the C-fiber-evoked potentials in SNL rats but LTD in the sham-operated rats. (A) LTP in SNL rats. (B) LTD in sham-operated rats. Twelve test stimuli (10–20 V, 0.5 ms, delivered every 5 min) were applied to sciatic afferents during a 60-min period before EA. Post-EA responses were recorded every 5 min for up to 2 h. Note that 100 Hz EA produced a significant LTP of the C-fiber-evoked potentials in SNL rats (A), but LTD in the sham-operated rats (B). Two individual potentials recorded in an animal are shown in inlets. Trace in (a) is an example of a recording before EA, trace in (b) was recorded after EA.

receptor antagonist bicuculline or non-selective 5-HT receptor antagonist methysergide. As a control, MK-801 or naloxone given alone had no significant effect on the C-fiber-evoked potentials (data not shown). These results indicate that the 2 Hz EA-induced spinal LTD was dependent on the activation of NMDA receptors and opioid receptors, but not GABA-A or 5-HT receptors. These results further provide an electrophysiological basis for our earlier reports that naloxone could block the analgesic effects of 2 Hz EA on neuropathic pain (Han, 2001; Sun et al., 2004). Our previous data suggested that 2 Hz EA elicits the release and activates the synthesis of the endogenous opioid peptides (e.g., endorphin, enkephalins, and endomorphin) (Guo et al., 1996a,b; Han, 2003, 2004). Therefore, we believe that the analgesic effects of 2 Hz EA depend on the induction of the NMDA receptor-dependent LTD via activation of the endogenous opioid peptides system.

Another interesting finding in the present study was that 100 Hz EA induced LTD of the C-fiber-evoked potentials in sham-operated rats, but LTP in SNL rats. The 100 Hz EA-induced LTD in sham-operated rats could be blocked only by a combined pretreatment of bicuculline and methysergide. These results suggested that the high-frequency EA-induced LTD in normal rats was very different from the low-frequency EA-induced LTD in SNL rats. The former was another form of heterosynaptic LTD, which was dependent on the tonic endogenous inhibition, probably the endogenous GABAergic and serotonergic inhibitory system (Liu et al., 1998; Ikeda et al., 2000; Garraway and Hochman, 2001), whereas the latter was dependent on NMDA receptors and the endogenous opioid peptide system (Sandkuhler et al., 1997; Ikeda et al., 1999; Sandkuhler, 2000). It is unclear why 100 Hz EA induces spinal LTP in the SNL rats, but a possible explanation may result from the disinhibition of function of the tonic endogenous inhibitory system (Woolf and Salter, 2000; Zimmermann, 2001; Malan et al., 2002; Woolf, 2004; Gwak et al., 2006) due to the loss of inhibitory GABA functions (Moore et al., 2002; Miletic et al., 2003; Rode et al., 2005; Scholz et al., 2005; Gwak et al., 2006) and/or serotonin functions (Hains et al., 2001, 2002, 2003a,b; Nitanda et al., 2005; Honda et al., 2006).

As discussed above, spinal LTP played very important roles in the development and maintenance of neuropathic pain. Taken together the present findings with our previous data, we conclude that different modulation of EA on LTP or LTD might have different analgesic effects on neuropathic pain. It is also suggested that, if spinal synaptic LTP was induced, then neuropathic pain occurred like in the situation in SNL rats. On the contrary, if LTD was evoked instead of LTP, then neuropathic pain was attenuated like in the situation of EA at 2 Hz application in SNL rats. Therefore, the direction of the long-term synaptic plasticity like LTP or LTD in spinal dorsal horn might determine the development or prohibition of neuropathic pain.

In summary, our results demonstrate that: (1) the long-term synaptic plasticity in the spinal dorsal horn may play a key role in the generation of neuropathic pain and may be the underlying mechanism of the frequency-dependent analgesic effects of EA stimulation. (2) The 2 Hz EA-induced LTD in the spinal dorsal horn may be a potential mechanism for the analgesic effects of low-frequency EA on neuropathic pain. The less potent analgesic effect of high-frequency EA may result from the inability to induce LTD of the synaptic transmission in the spinal dorsal horn. (3) The high-frequency EA-induced LTD was different from the low-frequency EA-induced LTD. The former was dependent on the tonic endogenous inhibition, probably the endogenous GABAergic and serotonergic inhibitory system, whereas the latter was dependent on the NMDA receptors and the endogenous opioid peptide system. (4) The high-frequency EA induced spinal LTD in the sham-operated rats but LTP in SNL rats may result from the loss of the tonic endogenous inhibitory system (disinhibition) after nerve injury.

Conclusions

(1) Hyperexcitability like LTP in the spinal nociceptive synaptic transmission might occur after nerve injury, which might underlie the development of neuropathic pain. (2) LTD of

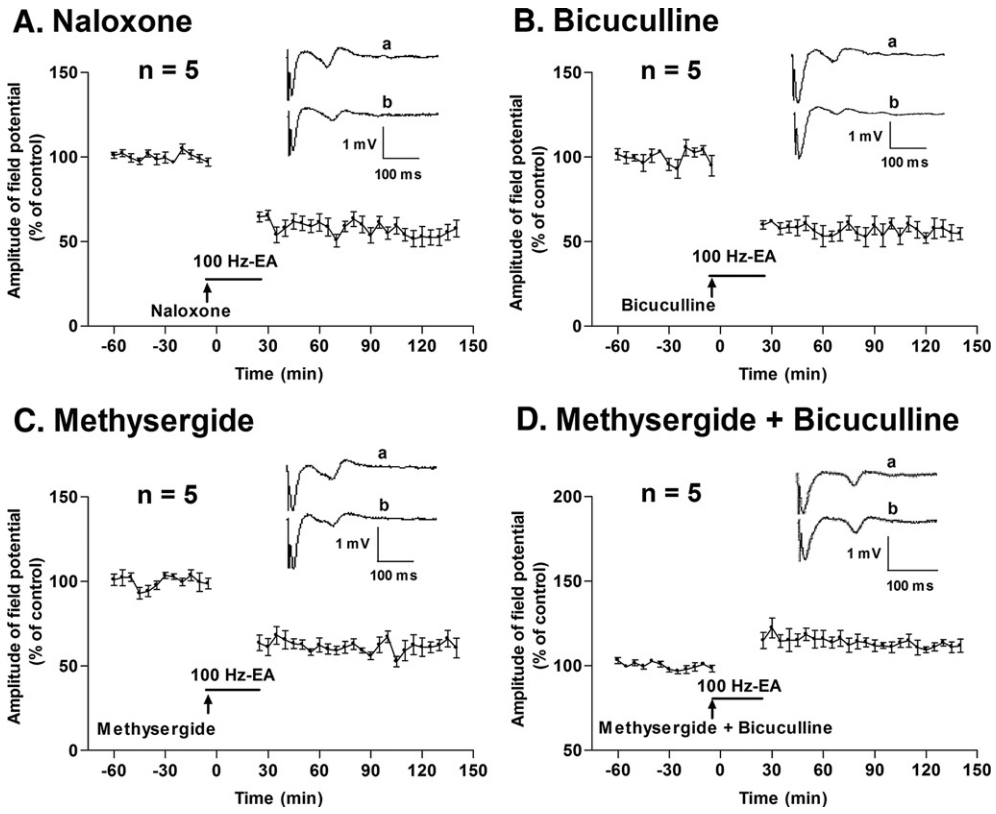

Fig. 6. 100 Hz EA-induced LTD in the sham-operated rats was blocked by a combination of bicuculline with methysergide. The EA-induced LTD could not be blocked by naloxone (A), bicuculline (B) or methysergide (C) respectively, but by a combination of bicuculline with methysergide (D). The induction of LTD was not only blocked, but also converted into LTP (D). Two individual potentials recorded in an animal are shown in inlets. Trace in (a) is an example of a recording before EA and trace in (b) was recorded after EA.

synaptic strength in spinal dorsal horn might be a potential mechanism underlying the differential antinociceptive effects of EA at low-frequency (like 2 Hz) and high-frequency (like 100 Hz) stimulation on neuropathic pain. Therefore, the direction of the long-term synaptic plasticity like LTP or LTD in spinal dorsal horn might determine the development or prohibition of neuropathic pain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.expneurol.2007.09.004.

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