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Ketamine potentiates the effect of electroacupuncture on mechanical allodynia in a rat model of neuropathic pain

Cheng Huang[a,b], Han-Ting Li[b], Yu-Shun Shi[a], Ji-Sheng Han[a], You Wan[a,*]

[a] *Key Laboratory of Neuroscience, Neuroscience Research Institute, Peking University, Ministry of Education, 38 Xueyuan Road, Beijing 100083, PR China*
[b] *Department of Physiology, Gannan Medical College, Ganzhou 341000, PR China*

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Abstract

Mu-opioid agonists and *N*-methyl-D-aspartate (NMDA) receptor antagonists have been shown to attenuate mechanical allodynia in neuropathic pain models. We have previously reported that 2 Hz electroacupuncture (EA) produced analgesia via releasing endogenous opioid peptides (i.e. β-endorphin and endomorphin) and the activated μ-opioid receptors. The present study aimed to examine whether ketamine, an NMDA receptor antagonist, can enhance the anti-allodynic effects induced by 2 Hz EA in a rat model of neuropathic pain following spinal nerve ligation (SNL). The results are as follows: (1) EA itself or i.p. injection of ketamine reduced mechanical allodynia (i.e. increase in withdrawal threshold). (2) Although injection of ketamine at a low dose (1.0 mg/kg) alone did not influence mechanical withdrawal threshold, combination of ketamine at this dose with EA produced more potent anti-allodynic effect than that induced by EA alone. (3) The anti-allodynic effect of EA combined with ketamine could be reversed by i.p. injection of naloxone (2.0 mg/kg). These results suggested that ketamine potentiate the anti-allodynic of EA in rats with spinal nerve ligation, and endogenous opioid system is likely to be involved in this process.

Keywords: Neuropathic pain; Allodynia; Ketamine; NMDA receptor; Electroacupuncture; Opioid peptide

Neuropathic pain resulting from nerve injury is characterized by spontaneous pain, allodynia (the perception of normally innocuous stimuli as painful) and hyperalgesia (an increased sensitivity to painful stimuli) [3,14]. We and others have shown that activation of the *N*-methyl-D-aspartate (NMDA) receptors is important in the initiation and maintenance of persistent pain following nerve injury [27,29]. Systemic administration of NMDA receptor antagonists is effective in the treatment of neuropathic pain in humans and animals [9,22,25,28]. However, the clinical application of the currently available NMDA receptor antagonists was hindered by their side effects [9].

Although the efficacy of opioids, for example morphine, in the management of neuropathic pain was debated, recent studies indicated that exogenously administration of endomorphin, a μ-opioid receptor agonist, significantly reduced mechanical allodynia in rats following nerve injury, and this effect was more potent than that of morphine [24]. Our previous studies indicated that 2 Hz electroacupuncture (EA) produced anti-nociception via the release of endomorphin which activates μ-opioid receptor in normal rats and mice [11,12]. NMDA receptor antagonists have been shown to potentiate opioid anti-nociception in animals [5]. We also observed that ketamine, an NMDA receptor antagonist, could significantly enhance EA-induced anti-nociception in normal rats (unpublished data). The present study aims to test the effects of combination of EA with ketamine in reducing mechanical allodynia in a rat model of neuropathic pain.

Female Sprague–Dawley rats weighing 200–250 g were provided by the Animal Department of Peking University Health Science Center. They were housed four to five per cage with food pellets and water ad libitum. In all experiments, measures were taken to minimize pain and/or discomfort. All

* Corresponding author. Tel.: +86 10 8280 1151; fax: +86 10 8280 1111.
E-mail address: ywan@bjmu.edu.cn (Y. Wan).

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experiment procedures were approved by the Animal Use and Protection Committee of our university and in accordance with the NIH Guide for the Care and Use of lab Animals. Ketamine hydrochloride and naloxone hydrochloride dihydrate were products of Sigma Chemicals Company (USA). Ketamine or naloxone was dissolved in normal saline (NS) and injected intraperitoneally (i.p.) in a volume of 1 ml/kg.

Neuropathic pain model was established by L5–L6 spinal nerve ligation (SNL) according to the procedures of Kim and Chung [14]. Rats were anesthetized with 10% chlorohydrate (0.3 ml/100 g body weight) and placed in the prone position to access the right L5–L6 spinal nerves. The dorsal vertebral column from L5 to L6 spinal nerves were exposed and was tightly ligated distally to the dorsal root ganglion using 4–0 silk sutures. Animals were allowed to recover for 5 days. The rats exhibited motor deficiency or no tactile allodynia were excluded from further experiments.

Rats were restrained in specially designed holders with their hind legs and tails exposed as described in our previous report [11]. Two stainless-steel needles with 0.4 mm diameter, 4 mm length were inserted into each leg. One needle was at the Zusanli acupoint (ST36), which was 5 mm lateral to the anterior tubercle of the tibia marked by a notch, and the other needle was at the Sanyinjiao acupoint (SP6), which was 3 mm proximal to the medial melleolus and at the posterior border of the tibia. Square waves of EA generated from a Han's Acupoint Nerve Stimulator (HANS, LH series, manufactured in our university) were applied to both legs simultaneously. The EA frequency of EA was 2 Hz with pulse width 0.6 ms, and the intensity was increased in a stepwise manner at (0.5–1.0–1.5) mA, each lasting for 10 min.

The mechanical withdrawal threshold was determined with the method described by Chaplan et al. [8] with minor modification. Briefly, a rat was placed in an individual plexiglass housing (18 cm × 8 cm × 8 cm) with wire mesh floor, and allowed to explore and groom until they settled down. A set of von Frey filaments (Stoeling Company) with bending forces ranging from 0.4 to 15.1 g were applied in an ascending order to the plantar surface of the right hind-paw. Hind-paw withdrawal was considered as positive response. The stimulation with one filament was repeated five times at 10–15 s interval. When lack of a response, the next filament with greater bending force was applied. The bending force of the von Frey filaments triggering the withdrawal of the hind-paw was recorded for three times and the average value from the three measurements was considered as the mechanical withdrawal threshold of the paw.

The experimental data were expressed as mean ± S.E.M., and analyzed with non-parametric one-way or two-way analyses of variance (ANOVA) where appropriate, and followed by Dunn's post-hoc test when needed. $P < 0.05$ was taken as statistically significant.

To observe the effect of ketamine on mechanical allodynia, rats with spinal nerve ligation were randomly divided into five groups ($n = 7$ per group) of NS and ketamine at 0.3, 1.0, 3.0, and 10.0 mg/kg. Before administration of ketamine or NS, basal mechanical allodynia was measured with von Frey filaments. Ketamine or NS was injected intraperitoneally (i.p.) once a day from day 1 to day 6 (08:00–10:00 a.m.) in each week for 4 weeks. The mechanical withdrawal thresholds were measured again on day 7 of weeks 1–4 after injection of ketamine or NS. Results are shown in Fig. 1. The mechanical withdrawal thresholds in groups of ketamine 3.0 or 10.0 mg/kg, but not of ketamine 0.3 or 1.0 mg/kg, increased significantly at weeks 2–4 compared with those in NS group ($P < 0.01$).

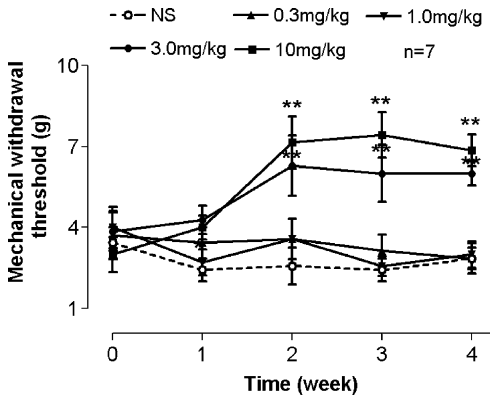

Fig. 1. The effects of ketamine on the mechanical allodynia in rats with spinal nerve ligation. Before treatment with ketamine or NS (at week 0), the mechanical withdrawal thresholds were measured as basal. The mechanical withdrawal thresholds were measured again at weeks 1–4 after administration with ketamine (0.3, 1.0, 3.0, and 10.0 mg/kg) or NS. Statistical analysis was carried out by non-parametric two-way analyses of variance (ANOVA) followed by Dunn's post-hoc test when needed. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with NS group at the corresponding time-points.

The effect of co-administration of EA and a low dose of ketamine on mechanical allodynia was shown in Fig. 2. Rats with neuropathic pain were randomly divided into four groups of NS ($n = 7$), ketamine (1.0 mg/kg) ($n = 8$), NS plus EA ($n = 8$) and ketamine (1.0 mg/kg) plus EA ($n = 8$). Ketamine (1.0 mg/kg) were injected once a day from day 1 to day 6 (08:00–10:00 a.m.) of each week for 4 weeks, EA was given to rats at day 3 and day 6 (10:00–12:00 a.m.) of each week for up to 4 weeks, respectively. The effects of EA or ketamine plus EA on mechanical allodynia were determined on day 7 (10:00–12:00 a.m.) of each week. The basal mechanical withdrawal thresholds were measured prior to injection of ketamine or the EA application. Ketamine plus EA or NS plus EA significantly increased the mechanical withdrawal thresholds at weeks 2–4 after treatment compared with NS ($P < 0.01$). In addition, ketamine plus EA was more potent than NS plus EA ($P < 0.05$). Ketamine (1.0 mg/kg) alone did not have any effects on mechanical withdrawal thresholds ($P > 0.05$) (Fig. 2).

In order to evaluate the possible involvement of endogenous opioids in the inhibitory effect of EA or ketamine plus EA on mechanical allodynia, naloxone blockade experiment was conducted. Naloxone (2.0 mg/kg) or NS was injected i.p. 15 min before EA application or injection of ketamine. The mechanical withdrawal thresholds were measured prior

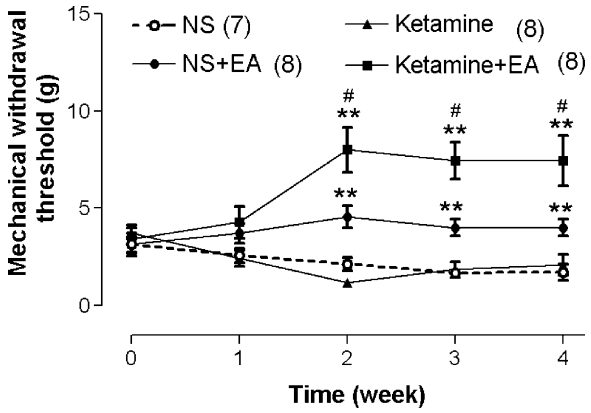

Fig. 2. The effect of combined administration of ketamine at low dose with EA on the mechanical allodynia in rats with spinal nerve ligation. Before treatment with ketamine or EA (at week 0), the mechanical withdrawal thresholds were measured as basal, the mechanical withdrawal thresholds were measured again at weeks 1–4 after administration of ketamine (1.0 mg/kg) or EA application, respectively. Statistical analysis was carried out by non-parametric two-way analyses of variance (ANOVA) followed by Dunn's post-hoc test when needed. The analgesic effect in groups of NS plus EA and ketamine plus EA were much more potent compared with that in groups of ketamine alone or NS at the corresponding time points, respectively ($^{**}P < 0.01$). The analgesic effect in the group of ketamine plus EA was more potent compared with that in the group of NS plus EA at the corresponding time points ($^{\#}P < 0.05$).

to EA and at 20, 40, 60, 80 min after EA. As shown in Fig. 3, naloxone significantly blocked the inhibitory effect of EA on mechanical allodynia at the time point of 20 min when compared to the NS group ($P < 0.01$). Similar effect was obtained at the time points of 20 or 40 min in ketamine plus EA group. This effect of naloxone was diminished 60 min after EA termination in both groups.

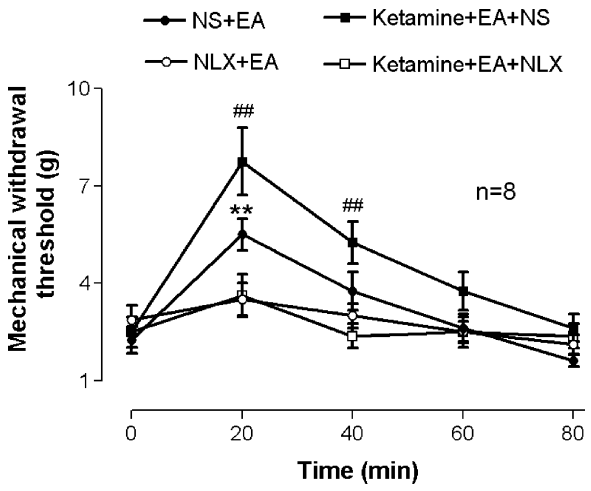

Fig. 3. Naloxone (NLX) blockade on the inhibitory effects of EA or ketamine plus EA on the mechanical allodynia in neuropathic pain rats. The basal mechanical withdrawal thresholds were evaluated prior to either EA application or ketamine administration. Rats received i.p. injection of either naloxone (2.0 mg/kg) or NS 15 min before treatment with EA or ketamine. The mechanical withdrawal thresholds were measured again 20, 40, 60, 80 min after treatment with EA or ketamine. The analgesic effect of NLX plus EA decreased significantly compared with NS plus EA group ($^{**}P < 0.01$), and the analgesic effect of ketamine plus EA plus NLX also decreased significantly when compared with that of ketamine plus EA plus NS group ($^{\#\#}P < 0.01$).

In the present study, we reported that EA decreases mechanical allodynia in a rat model of neuropathic pain, and that ketamine also dose-dependently inhibit allodynia. Furthermore, a low dose of ketamine potentiates the anti-allodynic effect of EA, an effect that is likely mediated by the endogenous opioid systems.

Acupuncture has long been used in China and other oriental countries in the treatment of many diseases including pain with few side effects [26,32]. Clinically, chronic pain is more complicated and common than acute pain. It is thus of interest to explore the analgesic effect of EA on chronic pain. Our present results showed that EA significantly decreased mechanical allodynia in rats with spinal nerve ligation, which is consistent with our previous results [27] and that of others [13].

It is well established that NMDA receptor plays an important role in the development and maintenance of neuropathic pain [6,16,22,27]. NMDA receptor antagonists, such as MK-801, were effective in relieving neuropathic pain in humans and animals model [22,27,28]. In the present study, we also observed that ketamine dose-dependently inhibited mechanical allodynia. We used repeated ketamine injection for a relatively long period of time and no significant effect was observed until after 2 weeks of continuous treatment. This suggested that a relatively long-term ketamine treatment regimen produced a cumulative therapeutic effect. Although the duration of the analgesic effect of ketamine is mostly brief, long lasting (hours) effect of ketamine has been reported in some patients with neuropathic pain [25]. Burton et al. [7] also reported mechanical allodynia was significantly reduced for at least 2 weeks after intrathecal ketamine was preemptively administered to animals undergoing surgery to induce neuropathic pain.

Ketamine is a weak antagonist of NMDA receptor that is often associated with severe side effects at high doses [22]. As mentioned above, EA is effective in the treatment of chronic pain, but the effect is mostly moderate. In the present study, we found that low dose of ketamine significantly enhanced the anti-allodynic effects of EA in nerve-injured rats. This result is similar with our observation that ketamine (5.0 mg/kg) could increase the anti-nociceptive effect induced by 100 Hz EA in normal rats (unpublished data). Such interaction between ketamine and EA is similar to that established between NMDA receptor antagonists and exogenous opioids. For example, Mao et al. [17] found that i.p. injection of dextromethorphan (3 mg/kg), an NMDA receptor antagonist, greatly enhanced anti-nociception and the duration of anti-nociception produced by s.c. injection of 5 mg/kg morphine. This result provides an option for the clinical strategy of treating neuropathic pain. However, there were also reports that ketamine attenuated the anti-nociceptive effect of EA [30,31]. We believed this may be due to methodological differences. In our work, we observed that ketamine (5 mg/kg)

potentiated the anti-nociceptive effect of 100 Hz EA in normal rats with stimulation of acupoints Sanyinjiao (SP6) and Zusanli (ST36), whereas others have used Changqiang and Yaoshu acupoints and reported that 4 or 20 mg/kg ketamine antagonized the effect of EA [30,31].

NMDA and opioid receptors interact in a complex fashion in the processing of nociception and in anti-nociception [20,23], For example, activation of NMDA receptor could decrease the effect of opioids in producing analgesia. However, repeated administration of opioids could mimic ongoing nociceptive inputs through interactions between opioid and NMDA receptors [18,19]. NMDA receptors activation in neuropathic pain and interactions between NMDA and opioid receptor systems changes the responsiveness to opioid analgesics [21]. Opioids also contributed to the neuronal plastic changes via interactions with NMDA receptors. Thus, for management of pathological pain, it is preferable to combine opioids with NMDA receptor antagonists. From the point of view of the opioid-NMDA interaction in pain or analgesia, our results support the concept that NMDA receptor system counteracts the anti-nociceptive effect of opioid system.

The present study also investigated the possible involvement of endogenous opioid systems in EA analgesia and in the potentiation by ketamine of EA analgesia with naloxone blockade experiments. As shown in Fig. 3, opioid receptor antagonist naloxone block the effect of EA or the ketamine plus EA on attenuating mechanical allodynia. Exogenously administration of opioid has anti-nociceptive effect in acute or chronic pain. Kim et al. [15] reported that direct spinal injection of morphine was effective in controlling central pain following spinal cord injury and either i.c.v. or systemic administration of morphine was effective in controlling mechanical allodynia [4] following L5/L6 nerve ligation. Similarly, the endogenous opioids released by EA also produces anti-nociception [10,12]. So it is not surprising that naloxone at the dose of 2.0 mg/kg could block the effects of EA. The more interesting finding from this experiment is that naloxone could also block the potentiation of low dose ketamine on the analgesic effects of EA on neuropathic pain. Although there was no direct evidence that naloxone (2.0 mg/kg) reversed the effect of ketamine, other studies have reported that ketamine produced a direct anti-nociceptive effect in rats via the opioid receptors [1,2]. Based on the above discussion, we speculated that the potentiation of ketamine on EA anti-allodynia in neuropathic pain may also be mediated by the activation of endogenous opioid receptor system.

Acknowledgments

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