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ORIGINAL PAPER

Electroacupuncture Effects in a Rat Model of Complete Freund's Adjuvant-Induced Inflammatory Pain: Antinociceptive Effects Enhanced and Tolerance Development Accelerated

Cheng Huang · Zhi-Qin Huang · Zhi-Ping Hu · Shao-Zu Jiang · Han-Ting Li · Ji-Sheng Han · You Wan

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Abstract We have previously shown that electroacupuncture (EA) produced antinociception through the release of endogenous opioid peptides to activate opioid receptors during acute nociception. EA produced tolerance after its prolonged application. It has reported that 100 Hz EA could reduce mechanical hyperalgesia in complete Freund's adjuvant (CFA)-induced inflammatory nociception rats. The present study aims to investigate the antinociceptive effect of EA and the development of EA tolerance in chronic inflammatory nociception rats with CFA injection into the hind paw plantar. The results showed that the antinociceptive effect of 100 Hz EA was significantly enhanced in CFA-induced inflammatory nociception rats. Naloxone at 20 mg/kg could significantly block this antinociceptive effect. Chronic tolerance to EA was developed faster in CFA-induced inflammatory nociception rats than in normal rats. Therefore, 100 Hz EA could enhance antinociceptive effects and accelerate tolerance development in CFA-induced inflammatory nociception rats. The enhancement of EA antinociceptive effect in CFA-induced inflammatory nociception rats might involve the endogenous opioid peptides such as dynorphin.

Keywords Electroacupuncture · Antinociception · Complete Freund's adjuvant · Inflammatory pain · Tolerance · Naloxone

Introduction

A great number of studies reported that electroacupuncture (EA) or peripheral electrical stimulation at specific frequencies triggered release of different kinds of endogenous opioid peptides in the central nervous system (CNS) [7]. For example, our previous studies found that 2 Hz EA mainly releases β-endorphin, endomorphin, and met-enkephalin, which take effects through activation of μ- and δ-opioid receptors. One hundred Hz EA produced antinociception through the release of dynorphin which then activates κ-opioid receptors in normal rats and mice [7]. However, this antinociceptive effect will decrease after prolonged 2 or 100 Hz EA application, which is called "EA tolerance" [7, 20].

Inflammatory pain is very common in clinical practice. Patients mainly suffer from ongoing pain (spontaneous pain), evoked pain, and hyperalgesia. Opioids are effective in treatment of inflammatory pain. For example, i.c.v. administration of μ-opioid receptor agonist DAMGO or morphine, or κ-opioid receptor agonist dynorphin significantly reduced hyperalgesia [1, 2]. In our previous report, 100 Hz EA could attenuate mechanical hyperalgesia in complete Freund's adjuvant (CFA)-induced inflammatory nociception, and this effect could be blocked by large dose of naloxone [9].

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C. Huang (✉) · Z.-P. Hu · S.-Z. Jiang · H.-T. Li
Department of Physiology, Gannan Medical University,
1 Yixueyuan Road, Ganzhou 341000, Jiang-Xi Province, China
e-mail: huangc9a@yahoo.com

C. Huang · J.-S. Han · Y. Wan (✉)
Neuroscience Research Institute, Peking University,
38 Xueyuan Road, Beijing 100083, China
e-mail: ywan@hsc.pku.edu.cn

Z.-Q. Huang
Department of Pharmacy, Gannan Medical University,
1 Yixueyuan Road, Ganzhou 341000, Jiang-Xi Province, China

However, under chronic inflammatory pain condition, contradictory results have been reported about the efficacy of analgesic effects of opioids and about the susceptibility to the development of opioid tolerance. For example, both μ- and κ-opioid receptor agonists exerted enhanced antinociceptive effects in chronic inflammatory nociception in arthritic rats [15]. Repetitive application of morphine at low doses produced tolerance rapidly in arthritic rats [11], although morphine failed to produce tolerance in rats with acute formalin inflammatory nociception [21].

EA antinociception and EA tolerance development has been studied extensively in normal rats, but little is known in rats with chronic inflammatory nociception. We previously reported that 100 Hz EA attenuated mechanical but not thermal hyperalgesia in CFA-induced inflammatory nociception, and this effect could be blocked by large dose of naloxone [9]. In the present study, we observed the EA antinociceptive effects and EA tolerance development in CFA-induced chronic inflammatory nociception rats. Furthermore, blockade experiment with naloxone at large doses was conducted to investigate the possible involvement of endogenous opioid peptides in the 100 Hz EA antinociception.

Experimental Procedures

Animals and Chemicals

Female Sprague–Dawley rats weighing 200–250 g were provided by the Department of Laboratory Animal Science of Peking University Health Science Center. They were housed 4–5 per cage with food pellets and water ad libitum according to the University Animal Care and Use Committee Guidelines adopted from NIH, USA. All possible measures were taken to minimize pain and/or discomfort. CFA and naloxone hydrochloride were products of Sigma Chemicals Company (USA). Naloxone was dissolved in normal saline (NS). Naloxone (20 mg/kg) or NS were injected intraperitoneally (i.p.) 20 min prior to EA. All injection was in a volume of 1.0 ml/kg.

Establishment of CFA-Induced Inflammatory Pain Model

CFA-induced inflammatory pain model was established according to our previous report [9]. Briefly, rats were anesthetized with 10% chlorohydrate (0.3 ml per 100 g body weight). One hundred microliter of CFA was injected into the plantar surface of the left hind paw using a syringe with a 28-gauge needle. Rats were kept at room temperature (22 ± 1°C) for recovery after CFA injection and further experiments were performed 48 h after CFA injection.

Electroacupuncture Application

Room temperature was maintained at 22 ± 1°C during experiment. EA was applied according to our routine procedure [17]. Briefly, each rat was gently placed into a specially designed polyethylene holder, with the hind legs and tail exposed. The skin of the hind legs of rats and acupuncture needles were sterilized with 75% alcohol. Stainless-steel needles (0.4 mm in diameter, 4 mm in length) were inserted into the "acupoints" in each hind leg. One acupoint was "Zusanli" (ST 36, 4 mm lateral to the anterior tubercle of the tibia, which is marked by a notch) and the other was "Sanyinjiao" (SP 6, 3 mm proximal to the medial melleolus, at the posterior border of the tibia). During EA application, rats were kept in the holder without any anesthetics. The stimuli were generated from an electric device named Han's Acupoint Nerve Stimulator (HANS, LH series) and applied to both legs simultaneously. The electric stimuli were set as square waves, 0.2 ms in pulse width, and 100 Hz in frequency. The intensities were increased in a stepwise manner at 1.0–1.2–1.5 mA, with each intensity lasting for 10 min.

Nociceptive Testing with Tail Flick Latency (TFL)

TFL was assessed using radiant heat as described previously [9, 17]. Focused light (3 mm in diameter) from a 12.5 W projection bulb was applied to the tail skin 3–4 cm from the tip, the TFL was measured to the nearest 0.1 s. The intensity of the thermal stimulus was adjusted by changing the voltage of electricity so as to the basal latency was within the range of 4–6 s. To avoid tissue damage, a cut-off limit of 15 s was used. Thirty minutes after rats were placed in the holder and acupuncture needles were inserted, the TFL was examined, and the mean of three consecutive measures at 5 min intervals was taken as the basal TFL. TFL was measured every 10 min during the 30 min EA application. The percent of TFL increase was taken as the EA-induced antinociception, as calculated as follows: TFL(%) = (latency after EA − basal latency)/basal latency × 100%.

Groups of Animals

In order to compare the antinociceptive effects of 100 Hz EA in normal rats and CFA-induced inflammatory nociception rats. Forty-eight hours after CFA injection, 20 rats were randomly divided into two groups: CFA group and CFA plus EA group. Twenty normal rats were also randomly divided into two groups: normal group and normal plus EA group. After assessment of the basal TFL, EA was applied for 30 min. The TFL was determined every 10 min during EA application, and the percent change was used to represent the extent of EA-induced antinociception.

For naloxone blockade experiment, 40 rats were randomly divided into four groups: control, EA, EA plus NS, and EA plus naloxone 48 h after CFA injection. Naloxone (20 mg/kg) or NS was injected i.p. 20 min before EA application.

Electroacupuncture Tolerance

EA chronic tolerance was developed according to our previous report [10]. EA was applied once daily, 30 min for each time for 6 days consecutively. The TFL was examined immediately after final EA application to evaluate the EA-induced antinociceptive effect. The antinociceptive effect decreased over the course of EA application, which indicated the development of chronic tolerance to EA [20].

Statistical Analysis

Data were expressed as mean ± SEM. Difference among groups was analyzed with two-way or one-way analyses of variance (ANOVA) where appropriate, followed by Newman–Keuls post hoc test. $P < 0.05$ was considered as statistically significant.

Results

Analgesic Effects of 100 Hz EA in CFA-Induced Inflammatory Nociception Rats

Antinociceptive effects of EA in normal rats and CFA-induced inflammatory nociception rats were observed. The results are shown in Fig. 1. EA-induced antinociception in normal rats and CFA-induced inflammatory nociception rats ($P < 0.01$). More importantly, EA produced a much stronger antinociceptive effect in the CFA-induced inflammatory nociception rats than that in normal rats ($P < 0.05$).

Development of Chronic Tolerance to 100 Hz EA in CFA-Induced Inflammatory Nociception Rats

As shown in Fig. 2, development of chronic tolerance to EA accelerated in CFA-induced inflammatory nociception rats. As stated above, to develop chronic tolerance to EA in normal or CFA-induced inflammatory nociception rats, EA was given once daily for 6 days. In normal animals, the antinociceptive effects of EA decreased significantly from day 4 on, indicating a development of EA tolerance. The antinociceptive effects decreased from 49.9 ± 5.7% on day 1 to 31.6 ± 3.7% on day 4 ($P < 0.01$). In CFA-induced inflammatory nociception rats, the EA antinociceptive effects also decreased significantly from 65.2 ± 5.0% on day 1 to 32.4 ± 2.1% on day 3 ($P < 0.01$). EA tolerance developed from day 3 on. These results suggest that tolerance to 100 Hz EA developed 1 day faster in the CFA-induced inflammatory nociception rats than in normal rats.

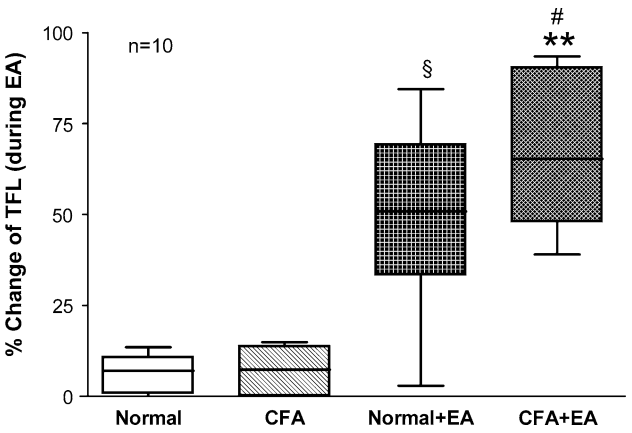

Fig. 1 The enhanced antinociceptive effect of 100 Hz EA on the CFA-induced inflammatory nociception rats. Twenty CFA-injected rats were randomly divided into two groups of CFA and CFA plus EA; 20 normal rats were also divided randomly into two groups of normal and normal plus EA. EA at 100 Hz was applied for 30 min, tail flick latency (TFL) was measured every 10 min during EA. Data are expressed as means ± SEM. $^{**}P < 0.01$ compared with the CFA group, $^{\S}P < 0.05$ compared with the normal group, $^{\#}P < 0.05$ compared with the normal plus EA group

Naloxone Blockade on the Analgesic Effect of 100 Hz EA in CFA-Induced Inflammatory Nociception Rats

In order to investigate the possible involvement of endogenous opioids in the antinociceptive effect of EA in CFA-induced inflammatory nociception, naloxone blockade

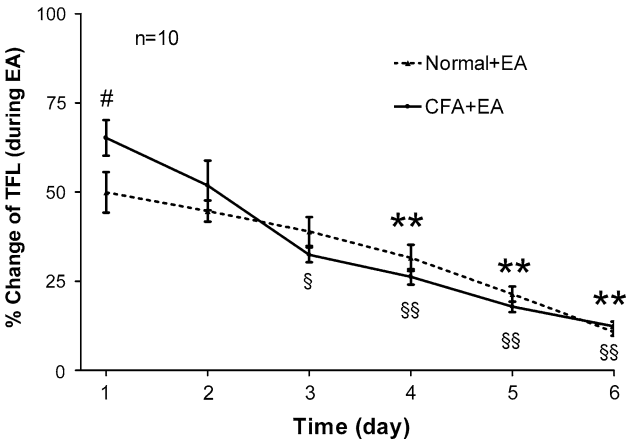

Fig. 2 Accelerated development of tolerance to 100 Hz EA in rats with CFA-induced inflammatory nociception. The normal and CFA-injected rats were given EA once daily for six successive days. Tail flick latency (TFL) was immediately measured after the final EA application. Data are expressed as means ± SEM. $^{**}P < 0.01$, $^{\S\S}P < 0.01$ compared to the first session EA in the corresponding group, $^{\#}P < 0.05$ compared with the normal plus EA group

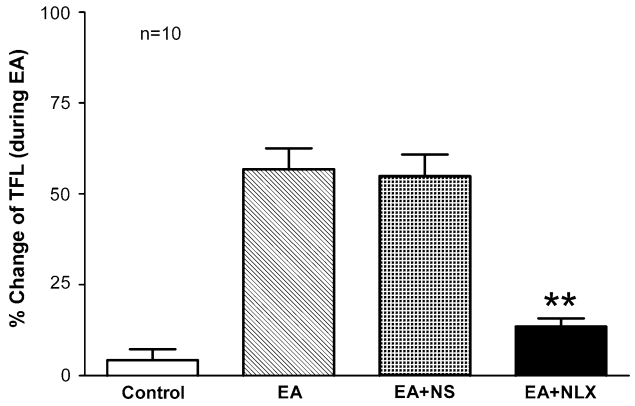

Fig. 3 Naloxone blockade on the enhanced antinociception induced by 100 Hz EA in the CFA-induced inflammatory nociception rats. Forty CFA-induced inflammatory nociception rats were randomly divided into four groups of control, EA, EA plus normal saline (NS) and EA plus naloxone (NLX). NLX (20 mg/kg) or NS was injected i.p. 20 min before EA application. Data are expressed as means ± SEM. $^{**}P < 0.01$ compared with the corresponding EA plus NS group

experiment was conducted. As shown in Fig. 3, naloxone significantly blocked EA-induced antinociceptive effects compared to the NS group ($P < 0.01$). This result indicates that endogenous opioid might be involved in the EA-induced antinociceptive effects in the CFA-induced inflammatory nociception rats.

Discussion

The present study demonstrated that the antinociceptive effects of 100 Hz EA were significantly enhanced under CFA-induced chronic inflammatory nociception state in rats (Fig. 1). This EA-induced antinociceptive effect could be blocked by naloxone at dose of 20 mg/kg (Fig. 3), indicating that endogenous opioid peptides especially dynorphin might be involved [7].

The mechanisms underlying EA antinociception are complicated. Different antinociceptive systems including opioid peptides are involved in normal animals [7]. It is known that exogenously i.t. injection of dynorphin produced antinociception through the activation of κ-opioid receptors in the spinal cord [8]. EA at 100 Hz accelerates the release of endogenous dynorphin in the spinal cord and produces antinociception in normal rats [7].

The effects of opioids have also been investigated in great detail in animal models of inflammatory nociception. For example, exogenous administration of opioid agonists showed antinociceptive activity under inflammatory conditions. κ-opioid receptor agonist dynorphin significantly reduced the peripheral inflammation-induced hyperalgesia [1], another κ-opioid receptor antagonist nor-BNI also produced a dose-dependent increase in arthritic flexion pain scores (pain-killing effect) [13]. These results suggest that dynorphin/kappa system had antinociceptive effects in arthritic pain animals.

It has been reported that endogenous opioid system changed during inflammatory pain. In CFA-induced polyarthritic rats, dynorphin synthesis increased in the lumbar spinal cord, which might play a role in nociception modulation [14]. Prodynorphin/preprodynorphin mRNA and dynorphin protein were up-regulated after CFA injection [1, 19]. Preprodynorphin mRNA increased parallel to the development of behavioral hyperalgesia. These suggest the enhanced opioid biosynthesis during chronic inflammatory pain [14]. In the present study, the enhanced antinociceptive effect of 100 Hz EA under the CFA-induced inflammatory nociception condition might result from the increased release of endogenous dynorphin from both 100 Hz EA stimulation and CFA-induced inflammatory nociception. Other reports support this finding. For example, antinociceptive effects of μ- and κ-opioid receptor activation increased in arthritic rats [15]. Sensitivity to morphine antinociception enhanced in a rat model of chronic inflammatory nociception and 10 of 12 strains of mice with CFA-induced inflammatory nociception [4, 12]. Of course, cytokines and other mediators during CFA-induced inflammatory nociception should be taken into consideration, because cytokines have antinociceptive effects and can modulate opioid antinociceptive potency [18].

It was reported that the required blockade dose of naloxone for 100 Hz EA-induced analgesia was at 20 mg/kg [7]. Goldstein et al. reported that the dose of naloxone blockade on κ-receptor was 20-fold higher than that on μ- and δ-receptors in vitro [5]. Moreover, our recent study also demonstrated that large dose of naloxone could block the inhibitory effect of single session EA on mechanical hyperalgesia in CFA-induced inflammatory nociception [9]. In the present study, large dose of naloxone (20 mg/kg) blocked the antinociceptive effect induced by 100 Hz EA in CFA-induced inflammatory nociception. This result suggests that opioid systems, especially dynorphin and κ-receptor system, might be involved in the 100 Hz EA antinociception in the CFA-induced inflammatory nociception rats as in normal rats as reported previously [5, 7].

Whether development of opioid tolerance in chronic pain state is delayed or accelerated has been contradictory. For example, Kayser et al. demonstrated that repeated administration of low doses of morphine (0.3–3 mg/kg) in CFA-induced inflammatory nociception rats developed tolerance, and the tolerance developed rapidly after one day of treatment [11]. Gutstein et al. found that chronic nociceptive stimulus significantly accelerated the development of tolerance to morphine antinociception in CFA-induced inflammatory nociception rats [6]. Liang et al. provided evidence that nine strains of mice with

CFA-induced inflammatory nociception showed enhanced tolerance after morphine treatment compared to the control ones [12]. However, less tolerance in CFA-induced inflammatory nociception rats was also reported when the opioid antinociceptive bezitramide was chronically administered [3]. Other studies reported that no delaying effect in the development of opioid tolerance in acute formalin pain model [16, 21]. Development of chronic tolerance to 100 Hz EA was reported previously [6, 11, 12]. In the present study, acceleration of this chronic tolerance in CFA-induced inflammatory nociception rats was observed (Fig. 2). This indicates that in chronic inflammatory nociception states, endogenous opioids like dynorphin motivated by EA application might induce enhanced opioid tolerance.

In summary, the present study found that the antinociceptive effect of 100 Hz EA was enhanced and development of tolerance to 100 Hz EA was accelerated in rats with CFA-induced inflammatory pain. Endogenous opioids, especially dynorphin, might be involved in the EA antinociceptive effect in CFA-induced inflammatory nociception rats.

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References

1. Beyer A, Schafer M, Stein C (1997) Antinociceptive effects of dynorphin peptides in a model of inflammatory pain. Pain 70:141–147
2. Cabot PJ, Carter L, Schafer M, Stein C (2001) Methionine-enkephalin and dynorphin a release from immune cells and control of inflammatory pain. Pain 93:207–212
3. Colpaert FC (1979) Can chronic pain be suppressed despite purported tolerance to narcotic analgesia? Life Sci 24:1201–1209
4. Cook CD, Nickerson MD (2005) Nociceptive sensitivity and opioid antinociception and antihyperalgesia in Freund's adjuvant-induced arthritic male and female rats. J Pharmacol Exp Ther 313: 449–459
5. Goldstein A, Tachibana S, Lowney LI, Hunkapiller M, Hood L (1979) Dynorphin-(1–13), an extraordinarily potent opioid peptide. Proc Natl Acad Sci USA 76:6666–6670
6. Gutstein HB, Trujillo KA, Akil H (1995) Does chronic nociceptive stimulation alter the development of morphine tolerance? Brain Res 680:173–179
7. Han JS (2003) Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci 26:17–22
8. Han JS, Xie GX, Goldstein A (1984) Analgesia induced by intrathecal injection of dynorphin B in the rat. Life Sci 34:1573–1579
9. Huang C, Hu ZP, Long H, Shi YS, Han JS, Wan Y (2004) Attenuation of mechanical but not thermal hyperalgesia by electroacupuncture with the involvement of opioids in rat model of chronic inflammatory pain. Brain Res Bull 63:99–103
10. Huang C, Long H, Shi YS, Han JS, Wan Y (2005) Ketamine enhances the efficacy to and delays the development of tolerance to electroacupuncture-induced antinociception in rats. Neurosci Lett 375:138–142
11. Kayser V, Neil A, Guilbaud G (1986) Repeated low doses of morphine induce a rapid tolerance in arthritic rats but a potentiation of opiate analgesia in normal animals. Brain Res 383:392–396
12. Liang DY, Guo T, Liao G, Kingery WS, Peltz G, Clark JD (2006) Chronic pain and genetic background interact and influence opioid analgesia, tolerance, and physical dependence. Pain 121:232–240
13. Liu HX (1999) Repeated 100 Hz TENS for the treatment of chronic inflammatory pain in rats: optimal parameters and possible neurochemical mechanisms. Prog Physiol Sci 30:35–37
14. Millan MJ, Czlonkowski A, Pilcher CW, Almeida OF, Millan MH, Colpaert FC, Herz A (1987) A model of chronic pain in the rat: functional correlates of alterations in the activity of opioid systems. J Neurosci 7:77–87
15. Neil A, Kayser V, Gacel G, Besson JM, Guilbaud G (1986) Opioid receptor types and antinociceptive activity in chronic inflammation: both kappa- and mu-opiate agonistic effects are enhanced in arthritic rats. Eur J Pharmacol 130:203–208
16. Rahman AF, Takahashi M, Kaneto H (1994) Involvement of pain associated anxiety in the development of morphine tolerance in formalin treated mice. Jpn J Pharmacol 65:313–317
17. Ren MF, Han JS (1979) Rat tail-flick acupuncture method. Chin Med J 92:576–582
18. Shavit Y, Wolf G, Goshen I, Livshits D, Yirmiya R (2005) Interleukin-1 antagonizes morphine analgesia and underlies morphine tolerance. Pain 115:50–59
19. Spetea M, Rydelius G, Nylander I, Ahmed M, Bileviciute-Ljungar I, Lundeberg T, Svensson S, Kreicbergs A (2002) Alteration in endogenous opioid systems due to chronic inflammatory pain conditions. Eur J Pharmacol 435:245–252
20. Tian JH, Zhang W, Fang Y, Xu W, Grandy DK, Han JS (1998) Endogenous orphanin FQ: evidence for a role in the modulation of electroacupuncture antinociception and the development of tolerance to antinociception produced by morphine and electroacupuncture. Br J Pharmacol 124:21–26
21. Vaccarino AL, Marek P, Kest B, Ben-Eliyahu S Jr, Couret LC, Kao B, Liebeskind JC (1993) Morphine fails to produce tolerance when administered in the presence of formalin pain in rats. Brain Res 627:287–290