Effects of electroacupuncture on the mechanical allodynia in the rat model of neuropathic pain

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Abstract

The analgesic effects of acupuncture on the mechanical allodynia in the rat model of neuropathic pain have not yet been studied. The aim of the present study is: first, to determine if electroacupuncture (EA) or morphine attenuates the mechanical allodynia; and secondly, to examine if the EA effect may be mediated by endogenous opioids. To produce neuropathic pain, the right superior caudal trunk was resected between the S3 and S4 spinal nerves. Twenty-one days after the neuropathic surgery, low frequency EA stimulation (2 Hz, 0.3 ms, 0.07 mA) delivered to Houxi (S13) for 30 min relieved significantly the signs of mechanical allodynia. Intraperitoneal (i.p.) morphine (0.5 or 1.5 mg/kg) also relieved the signs of mechanical allodynia in a dose-dependent manner. In addition, the antiallodynic effect of Houxi EA was blocked by pretreatment with naloxone (2 mg/kg, i.p.). However, combined application of EA and morphine did not show an obvious synergistic effect. These results suggest that low frequency EA or morphine can relieve the mechanical allodynia signs and the EA effect can be mediated by endogenous opioid systems.

Keywords: Electroacupuncture; Opioid; Naloxone; Neuropathic pain; Mechanical allodynia; Morphine

Partial peripheral nerve injury sometimes leads to neuropathic pain. This type of pain is characterized by spontaneous burning pain, hyperalgesia (an increased sensitivity to painful stimuli), and allodynia (the perception of normally innocuous stimuli as painful). Numerous studies have attempted to find pathophysiological mechanisms or drug effects on this abnormal sensation in patients or animals [3]. Acupuncture analgesia for this pain condition has been studied regarding neuropathic pain of malignancy [4], HIV-related peripheral neuropathic pain [7,12] and diabetic neuropathy in patients [5] or hyperalgesia in a neuropathic animal model [10].

However, although it has been well known that acupuncture analgesic effects on nociceptive pain are mediated by endogenous opioids and other neurotransmitters in the central nervous system [6], the mechanism of acupuncture analgesia for neuropathic pain is uncertain. Furthermore, it is controversial whether morphine relieves the neuropathic pain. Thus, the present study aims to determine whether electroacupuncture (EA) or morphine can relieve mechanical allodynia produced by peripheral nerve injury and, if EA relieved the mechanical allodynia, to see whether EA effects can be mediated by an endogenous opioid.

Young adult male Sprague–Dawley rats ($n=79$; 200–250 g) were housed in group cages (4–5 per cage) with water and food available ad libitum. The room was light/dark (08:00–20:00 h light, 20:00–08:00 h dark) controlled and kept at 21–24°C. Under sodium pentobarbital anesthesia (40 mg/kg, intraperitoneally (i.p.)), the right superior caudal trunk was exposed and transected at the level between the S3 and S4 spinal nerves that innervate the rat tail as previously described by Na and his coworkers [9]. Mechanical sensitivity was examined 1 day prior to the neuropathic surgery, and 1, 4, 7, 14 and 21 days postoperatively. As described previously [9], the mechanical allodynia was assessed by normally innocuous stimulation of the tail with the von Frey hair (bending force, 19.6 mN, 2.0 g). For proper application of stimuli with the von Frey hair and acupuncture, the rat was restrained in a plastic holder.
Each holder (5.3 × 15 cm in diameter × length) and the tail was laid on a plate. The mechanically sensitive area was first determined by rubbing various areas of the tail with von Frey hair. The actual test was performed by gently poking the most sensitive spot with the von Frey hair. An abrupt tail movement of more than 0.5 cm was considered to be an abnormal response attributed to mechanical allodynia. The stimulation was repeated ten times at 10–20 s intervals for each animal on each testing time or day (Fig. 1). The degree of response was expressed as a percentage of response frequency and was determined as follows:

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\text{Response frequency (\%) = \frac{\text{number of abnormal responses}}{10} \times 100}
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Two weeks after the neuropathic surgery, the behavioral test was performed with von Frey hair, and rats displaying well-developed behavioral signs of mechanical allodynia were selected. These rats were restrained in special holders. Each holder (5.3 × 15 cm in diameter × length) has a rectangular hole (7 × 2.5, 8 × 3 cm) on one side, a glass funnel inside to cover the rat’s head, and a small barrier was placed between the forelimbs of a rat. Rats were properly fitted in these holders with their tails protruding outside, and they were allowed to adapt to the environment for 30–90 min/day for 3–7 days. After the adaption period, a pair of stainless steel needles (interpolary distance, 3 mm) were inserted into the Houxi acupoint (SI3), which is located laterally behind the distal end of the 5th metacarpal bone of each rat. This point has been used in clinical practice for pain relief [13]. This method for stimulating the acupoint located in the forelimb of a rat is somewhat similar to those previously used for mice [11]. To obtain the control data, a non-acupoint was selected in the abdominal area. Train-pulses (2 Hz, 0.3 ms pulse width, 0.07 mA, produced by Nihon Kohden made in Japan) were delivered to the inserted needles at both acupoint (H-EA) and non-acupoint (NA-EA) for 30 min, and the needles were removed immediately following the simulation. The behavioral test was performed prior to and 15, 30, 45 and 75 min after the beginning of EA.

If EA had relieved the signs of mechanical allodynia, in order to determine whether this effect on mechanical alldynia was mediated by an endogenous opioid system, naloxone (Sigma; 2 mg/kg) was injected i.p. 20 min before the EA. In addition, to assess the effects of morphine or EA with morphine on mechanical alldynia, morphine (Myeng Mun Pharmaceutical Co., South Korea; 0.5 and 1.5 mg/kg, i.p.) was injected 10 min before the EA.

Data are expressed as means ± SEM. The significance of statistical differences were determined using Friedman’s rank test followed by Dunnett’s post-hoc test in a group and using the Mann–Whitney U-test between two groups. P < 0.05 was considered significant.

The effects of H-EA or NA-EA on the mechanical alldynia are shown in Fig. 2. In the NA-EA group (Fig. 2; open circles), the values prior to and 15, 30, 45 and 75 min after the beginning of EA were 97.1 ± 1.8, 92.9 ± 2.9, 91.4 ± 4.6, 94.3 ± 2.9 and 97.1 ± 1.8%, respectively. No statistically significant difference between the values before and after EA was detected (Friedman’s rank test). On the other hand, in the H-EA group (Fig. 2; closed circles), the values for response frequencies prior to and 15, 30, 45 and 75 min after the beginning of EA were 97.1 ± 1.8, 71.4 ± 4.0, 64.3 ± 7.2, 84.3 ± 2.9 and 91.4 ± 4.0%, respectively. The values at 15, 30 and 45 min after the beginning of H-EA were all significantly lower than the value before EA (Dunnett’s post-hoc test after Friedman’s rank test) and the maximal relieving effect of H-EA was shown at 30 min after the beginning of EA. These results indicate that the stimulation of acupoint, but not non-acupoint can relieve the signs of mechanical alldynia in the neuropathic animal model.

The results of naloxone pretreatment of the H-EA rats are shown in Fig. 3. Naloxone pretreatment (H-EA + Nx
group) reversed the antiallodynic effect of H-EA, while H-EA + saline (H-EA + SL) did not show such changes. These results suggest that the antiallodynic effect of H-EA can be blocked by naloxone.

The effects of morphine (0.5 and 1.5 mg/kg) or H-EA with morphine (H-EA + M) on the signs of mechanical allodynia are shown in Fig. 4A,B. In the 0.5 mg/kg morphine group (Fig. 4A; closed triangle), the value at 15 min after the injection was slightly lower than the value before the injection, whereas the values at 15, 30, 45 and 75 min after the injection in the 1.5 mg/kg morphine group (Fig. 4B; closed triangle) were significantly lower than the value before the injection (Dunnett’s post-hoc test after Friedman’s rank test). However, in the H-EA + M groups (M (0.5 mg) or M (1.5 mg); Fig. 4A,B), no statistically significant difference was detected between H-EA + M and H-EA + SL groups, except at 45 min following the beginning of EA in the H-EA + M (1.5 mg) group (Mann–Whitney U-test). These results suggest that morphine relieves the signs of mechanical allodynia in a dose-dependent fashion. However, combined application of H-EA and morphine did not show an obvious synergistic effect.

The results of the present study demonstrate that low frequency (2 Hz) H-EA or morphine can reduce the signs of mechanical allodynia, produced by partial injury of the nerves innervating the rat’s tail. This antiallodynic effect of EA is in line with the findings of Leem et al. [8], that acupuncture-like transcutaneous electrical stimulation reduced the enhanced mechanical responsiveness of the spinal neurons in a rat model of peripheral neuropathy induced by a tight ligation of L5–6 spinal nerves (SNL). In addition, in the present study, the antiallodynic effects of EA were blocked when naloxone pretreatment was used. Consistent with the present results, naloxone prevented reduction of pain responses induced by manual acupuncture on hyperalgesia in neuropathic rats with a chronic constriction injury in the sciatic nerve [10]. Taken together, the antiallodynic effects of acupuncture on mechanical allodynia may be mediated by endogenous opioids.

Although the efficacy of opioids in relieving neuropathic pain is a controversial issue [2], the present result agrees with the results of Chung and Na [1] that mechanical allodynia in the SNL model was relieved by systemic morphine in a dose-dependent manner. However, H-EA + M (0.5 mg/kg), contrary to our expectation, did not induce a synergistic effect on the antinociceptive effects of H-EA, although H-EA + M (1.5 mg/kg) induced slightly more antiallodynic effects than H-EA. This phenomenon is similar to the results of Takeshige [14] that combined application of acupoint
stimulation and morphine (0.5 mg/kg, i.p.) did not show a synergistic effect on nociceptive pain in rats.

The results of this study suggest that morphine can relieve the signs of mechanical allodynia and the relieving effects of low frequency (2 Hz) H-EA on the mechanical allodynia can be mediated by endogenous opioids.

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