Differences of Electroacupuncture-induced Analgesic Effect in Normal and Inflammatory Conditions in Rats

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Abstract: It has been reported by Stein et al. that the immune system and peripheral opioid receptors are involved in the control of pain accompanying inflammation. Electroacupuncture (EA) is used to relieve various kinds of pain. However, little is known about the effect of electroacupuncture analgesia (EAA) during hyperalgesia elicited by inflammation. The aim of the present study was to compare (1) the individual variation of EAA, (2) the durability of EAA, and (3) the effect of naloxone on EAA between normal rats and rats subjected to acute inflammatory pain. Carrageenan was subcutaneously administered by intraplantar (i.pl.) injection of the left hind paw to induce a nociceptive response. Nociceptive thresholds were measured using the paw pressure threshold (PPT). Rats received EA at 3 Hz in the left anterior tibial muscles for 1 hour after carrageenan injection. Naloxone was administered by intraperitoneal (i.p.) or i.pl. injection just before EA. EAA was elicited in 15 of 29 normal rats. These rats were divided into responders and non-responders. EAA in the responder group was almost completely antagonized by i.p. injection of naloxone. In contrast, in all the rats with carrageenan-induced inflammation, EAA was elicited, lasted for at least 24 hours after carrageenan injection, and was dose-dependently antagonized by i.pl. injection, but not significantly by i.p. injection of naloxone. It seems likely that the EAA in the rats with carrageenan-induced inflammation differs from that in normal rats, and these findings suggest that peripheral opioid receptors are involved in EAA during inflammatory conditions.

Keywords: Electroacupuncture; Analgesia; Hyperalgesia; Carrageenan; Inflammation.
Introduction

Numerous investigations of the mechanism underlying electroacupuncture analgesia (EAA) have been performed in humans and animals. The evidence indicates that EAA was reversed by naloxone, an opioid receptor antagonist (Mayer et al., 1977; He, 1987; Chen et al., 1996), and that the quantity of β-endorphin or enkephalin in the cerebrospinal fluid increased after electroacupuncture (EA) (Clement-Jones et al., 1979 and 1980; He, 1987). Endogenous opioids and descending pain inhibitory systems have therefore been considered to be involved in EAA. At present, EA is used to relieve various kinds of pain. However, little is known about the effect of EAA during inflammatory pain.

It has been shown that under inflammatory conditions, various types of immunocytes contain and produce opioid peptides (Sibinga and Goldstein, 1988; Stein et al., 1990) and the opioid-containing immune cells migrate preferentially to inflamed sites (Stein, 1995). Stein et al. (1990) have also shown that environmental stimuli such as cold water swim stress (CWS) in rats with unilateral hind paw inflammation elicit peripheral opioid receptor-mediated anti-nociception in the inflamed paw. The opioids are released locally from immunocytes during environmental stimuli and inhibit pain by interacting with peripheral opioid receptors in animals (Stein et al., 1990) and in humans (Stein et al., 1993). The local immune system and peripheral opioid receptors are thus involved in inflammatory pain control (Stein et al., 1993; Stein, 1995).

Therefore, it is possible that the effect of EA during inflammatory pain differs from that under normal conditions. No study has yet compared the effect of EAA during the normal state and inflammation-induced hyperalgesia. In this study, we compared the individual variation and durability of EAA and the effect of naloxone on EAA between normal rats and rats with carrageenan-induced inflammation.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 280–380 g were purchased from Japan SLC, Inc. The animals were kept at 24 ± 1°C and a relative humidity of 50–60%. Standard laboratory rodent food and tap water were available ad libitum. All experiments were conducted during the light phase of a 12/12-hour (7 am/7 pm) light-dark cycle. This study followed the National Institutes of Health (NIH) regulations for humane experimentation on animals, and the guidelines of the International Association for the Study of Pain.

Animals were treated in accordance with our Institutional Committee guidelines for the treatment of experimental animals.

Experimental Protocols

Experiment 1: In this experiment, 29 normal rats were divided into responder and non-responder groups according to the magnitude of the effect of EAA. The time course of paw pressure threshold (PPT) was compared within both groups before and after EA.
Experiment 2: To determine the involvement of opioid receptors in the central nervous system (CNS), we examined whether the opioid receptor antagonist naloxone given by intraperitoneal (i.p.) injection blocked the PPT elevations produced by EA in rats in the responder group. Rats in the responder group were randomly divided into two sub-groups, and rats received i.p. injection of naloxone (1.0 mg/kg) or equal volume of saline just before EA.

Experiment 3: This experiment was carried out to evaluate the effect of EA during hyperalgesia elicited by carrageenan-induced inflammation. EA was started immediately after measuring the PPT 3 hours after carrageenan injection.

Experiment 4: To determine the involvement of opioid receptors in the CNS, we examined whether naloxone given by i.p. injection also blocked the PPT elevations produced by EA during hyperalgesia elicited by carrageenan-induced inflammation. Rats received i.p. injection of naloxone (0.5, 1.0 and 2.0 mg/kg) or equal volume of saline just before EA.

Experiment 5: To determine the involvement of peripheral opioid receptors, we examined whether naloxone given by local intraplantar (i.pl.) injection also blocked the PPT elevations produced by EA during hyperalgesia elicited by carrageenan-induced inflammation. Rats received i.pl. injection (0.1 ml) of naloxone (0.6, 1.2 and 2.4 µg) or equal volume of saline just before EA.

**Induction of Inflammation**

In Experiments 3, 4 and 5, carrageenan (2%, 0.1 ml; Sigma, St. Louis, MO) was subcutaneously administered by i.pl. injection to the left hind paw of rats under ether anesthesia using a 26-gauge needle to induce inflammation.

**Algesiometry**

Nociceptive thresholds were evaluated using an Analgesy-meter (Ugo Basile). Rats were gently restrained under a soft cloth jacket and incremental pressure was applied to the dorsal surface of the left hindpaw. The pressure required to elicit paw withdrawal, the PPT, was determined. We used a cut-off threshold of 250 g to avoid tissue damage.

The mean of two consecutive measurements, separated by 2 minutes, was determined after a rest period of 15 minutes. PPT was determined 15 minutes before, just before, and 3, 4, 5, 7, 9 and 24 hours after the carrageenan injection. In Experiments 1 and 2, the same schedule was adopted, but additional determinations were made at 20-minute intervals for 1 hour after the end of EA.

**Electroacupuncture**

A pair of stainless steel needles 0.20 mm in diameter and 30 mm in length were inserted into Zusanli (ST36) and 5 mm from Zusanli (the left anterior tibial muscles). A 3 Hz biphasic
square wave pulse (0.1 ms pulse width) was delivered via the needles for a period of 60 minutes using a constant current programmed pulse generator. The intensity of EA was increased according to a schedule of 1-2-3 mA, for 20 minutes at each intensity. The intensity was sufficient to produce a rhythmic muscle contraction of the hind legs. The rats were gently restrained under a soft cloth jacket during PPT measurement and the EA procedure. Except for these times, rats were allowed to move freely in their cage.

**Drugs**

Naloxone (USP) (1 mg) dissolved in sterile saline (1 ml) was administered by i.p. injection just before EA. In Experiment 5, naloxone dissolved in sterile saline (0.1 ml) was administered by i.pl. injection into the inflamed paw just before EA. Drugs were administered under a brief ether anesthesia. Control animals received sterile saline just before EA.

**Data Analysis**

PPT data are given as raw values (means ± SD) or as a percentage of the maximum possible effect (%MPE) according to the following formula: %MPE = [(post-EA PPT − pre-EA PPT) / (cut-off PPT − pre-EA PPT)] × 100%. Data were analyzed using a multifactorial analysis of variance (ANOVA). When the ANOVA revealed a significant difference between the groups, the Tukey’s test was used for further analysis; p < 0.05 was considered as statistically significant.

**Results**

**Effect of EA on PPT in Normal Rats (Experiment 1)**

Figure 1 shows the effect of EA on PPT in normal rats. In this study, we grouped the rats with an increase in PPT of 10% or less into EAA non-effective (non-responder), and those with an increase of more than 10% into EAA effective (responder) types according to Murai et al. (1979). Of a total of 29 rats, 15 rats (51.7%) comprised the responder group (mean increase ratio was 17.2%) and 14 rats (48.3%) comprised the non-responder group (mean increase ratio was 3.7%).

In the responder group, PPT before EA was 74.0 ± 9.3 g. EA produced a significant elevation of PPT that reached a maximum (104.4 ± 17.8 g) immediately after the EA and returned to baseline within 20–60 minutes. In the non-responder group, PPT before EA was 71.9 ± 5.4 g. After the EA, no elevation of PPT was observed. A significant difference between the non-responder group and the responder group was observed immediately, 20 minutes and 40 minutes after the EA (p < 0.001, Fig. 1).

In addition, it was observed that a second and third EA also produced elevation of PPT in the responder group, but not in the non-responder group.
Effect of Naloxone Given by i.p. Injection on EAA in the Responder Group (Experiment 2)

Figure 2 shows the effect of naloxone on EAA in the responder group. In the EA + naloxone group, PPT before EA was 69.5 ± 4.3 g, which was not different from the values in the EA + saline group. PPT elevations elicited by EA were not influenced by prior i.p. injection of saline, however, PPT elevations were not observed in the EA + naloxone (1.0 mg/kg) group immediately and following the EA (p < 0.001). Thus, EAA in the responder group was clearly antagonized by i.p. injection of naloxone.

Effect of EA during Hyperalgesia Elicited by Carrageenan-induced Inflammation (Experiment 3)

Figure 3 shows the time course of analgesia produced by EA during inflammatory hyperalgesia. In the control group, PPT before carrageenan injection was 78.8 ± 13.1 g. Three hours after the carrageenan injection, a marked ipsilateral inflammatory response (swelling and redness) appeared and PPT decreased significantly (50.5 ± 12.6 g). Moreover, this decrease continued for 24 hours after carrageenan injection.

In the EA group, PPT decreased to almost the same level as the control group 3 hours after the carrageenan injection. However, PPT increased significantly (90.5 ± 15.6 g) immediately after the EA (mean increase was 16.4%) (p < 0.001). The PPT elevations produced by EA lasted at least 20 hours after the EA (24 hours after carrageenan injection).
Figure 2. Effect of naloxone given by i.p. injection on EAA in responder. n = 6 in each group. The results are expressed as mean ± SD. *p < 0.001 (EA + saline i.p. versus EA + naloxone i.p.).

Figure 3. Effect of EA during hyperalgesia elicited by carrageenan-induced inflammation. n = 10 in the control group; n = 7 in the EA group. The results are expressed as mean ± SD. *p < 0.001 (control versus EA).
Effect of Naloxone Given by i.p. Injection on EAA during Hyperalgesia Elicited by Carrageenan-induced Inflammation (Experiment 4)

Figure 4 shows the effect of naloxone (i.p.) on EAA during inflammatory hyperalgesia. In all groups, PPT decreased 3 hours after the carrageenan injection. In the EA + naloxone (0.5 mg/kg) group, PPT increased significantly immediately after the EA (107.5 ± 22.0 g) and the PPT elevations lasted at least 20 hours after the EA. In the EA + naloxone (1.0 mg/kg) group, PPT tended to increase immediately after the EA and thereafter compared to that of the control group, but did not increase to the level of the EA + naloxone (0.5 mg/kg) group. In the EA + naloxone (2.0 mg/kg) group, PPT elevations produced by EA were similar to that of the EA + naloxone (1.0 mg/kg) group. Thus, PPT elevations produced by EA tended to be dose-dependently attenuated by i.p. injection of naloxone compared to the EA + saline group, however, naloxone given by i.p. injection could not antagonize PPT elevations completely.

Effect of Naloxone Given by Local i.pl. Injection on EAA during Hyperalgesia Elicited by Carrageenan-induced Inflammation (Experiment 5)

Figure 5 shows the effect of naloxone (i.pl.) on EAA during inflammatory hyperalgesia. In the EA + naloxone (0.6 µg) group, PPT increased to the same level immediately after the EA and thereafter as the EA + saline group. In the EA + naloxone (1.2 µg) group, PPT tended to increase immediately after the EA. However, the PPT elevations were not as

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Figure 4. Effect of naloxone given by i.p. injection on EAA during hyperalgesia elicited by carrageenan-induced inflammation. n = 7 in the EA + saline i.p. group; n = 6 in all other groups. The results are expressed as mean ± SD.
remarkable as those of the EA + saline or the EA + naloxone (0.6 µg) groups. Moreover, significant differences were observed after the EA between the EA + naloxone (1.2 µg) and the EA + saline groups (p < 0.001). In the EA + naloxone (2.4 µg) group, PPT elevation produced by EA was the same level as that of the EA + naloxone (1.2 µg) group. Thus, PPT elevations produced by EA were dose-dependently antagonized by local i.pl. injection of naloxone from immediately after the EA (p < 0.001, p < 0.01). Naloxone per se did not have analgesic or hyperalgesic effect (data not shown).

**Discussion**

EA has been used to relieve various kinds of pain. It is well accepted that EA increases the nociceptive threshold in humans and animals by activating the endogenous opioid system (Murai *et al*., 1979; Takeshige *et al*., 1983; He, 1987; Chen *et al*., 1996; Tang *et al*., 1997; Tian *et al*., 1998). However, the analgesic effect of EA in rats with inflammatory pain has not been studied in detail. We compared the individual sensitivity and durability of EAA, and the effect of naloxone on EAA between normal rats and rats with carrageenan-induced inflammation. Among the normal rats that received EA, 15 of 29 showed EAA, so we could categorize them into responder and non-responder groups, consistent with other studies (Murai *et al*., 1979; Takeshige *et al*., 1983). In the responder group, EA produced a significant...
The elevation of PPT that reached a maximum immediately after the EA and returned to baseline within 20–60 minutes. The EAA in normal responder rats was blocked by i.p. injection of naloxone. These findings are consistent with previous studies (Mayer et al., 1977; Murai et al., 1979; Takeshige et al., 1983; Iguchi et al., 1985; He, 1987; Chen et al., 1996) and suggested that EA could release endogenous opioids to produce analgesia.

At least two possibilities have been inferred regarding the individual variation in EAA. The first is a defect of opioid receptors due to either an insufficient receptor population, or a low affinity for the opioid ligands. Peets and Pomeranz (1978) have reported that mice genetically deficient in opioid receptors showed a poor EA analgesia. However, some reports have shown that there was no significant difference in the concentration of opioid receptors and the content of endogenous opioids in various regions of the brain between the responder and non-responder groups (Murai et al., 1979; Takeshige et al., 1983). Recently, the role of inherited genetic factors in EA sensitivity in mice has been shown (Wan et al., 2001; Park et al., 2002).

The second possibility is an excessive activation of anti-opioid peptides. It has been shown that the individual variation in EAA depends on a functional balance between the opioid peptides and the anti-opioid peptides in the CNS (Han et al., 1986), and that functional reductions of the anti-opioid peptide cholecystokinin-8 (CCK-8) or orphanin FQ/nociceptin can convert low-responder rats into high-responders (Tang et al., 1997; Tian et al., 1998). Lee et al. (2002) have also shown that not only CCK secretion, but also the density of CCK receptors has an important relationship with the individual sensitivity to EAA because CCK-A receptors are expressed in non-responders much more than in responders.

In this study, we observed EAA in rats with carrageenan-induced inflammation. We found that EAA in the rats with inflammatory pain lasted longer than in normal responders. Sluka et al. (1998) and Gopalkrishnan and Sluka (2000) have reported that the effects of transcutaneous nerve stimulation (TENS) on hyperalgesia elicited by inflammation lasted for 24 hours, which is consistent with our results. In the present study, EAA in rats with inflammatory pain tended to be dose-dependently attenuated by i.p. injection of naloxone. However, the EAA was not completely blocked by i.p. injection of naloxone of the same dose or two-fold doses used in normal responder rats. In contrast, the EAA in rats with carrageenan-induced inflammation was completely blocked in a dose-dependent manner by i.pl. injection of naloxone. These findings suggest that not only opioid receptors in the CNS, but also peripheral opioid receptors are involved in EAA in rats with inflammatory pain.

In hyperalgesia elicited by carrageenan-induced inflammation, neurochemical evidence has shown that levels of opioid gene transcripts and peptides increase in dorsal horn neurons ipsilateral to the inflamed paw (Iadarola et al., 1988; Noguchi et al., 1992). The excitability and the receptive field size of the dorsal horn neurons also increase during inflammation (Kocher et al., 1987; Stanfa et al., 1992). Moreover, it has been shown that the localized inflammation of a rat’s hindpaw elicits an accumulation of immune cells containing opioid peptides and these peptides are released by environmental stimuli in the inflamed tissue, which may activate peripheral opioid receptors (Stein et al., 1990). Thus, the EAA in rats with inflammatory pain may differ from the EAA in normal rats due to these phenomena. In addition to the endogenous opioid mechanism in the CNS, the local immune system may be
involved in the EAA in rats with inflammatory pain. The mechanism of EAA in rats with carrageenan-induced inflammation is still not clear. Further study is needed to clarify the mechanism of EAA during peripheral inflammation.

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References


