Effect of varying frequency and duration of electroacupuncture stimulation on carrageenan-induced hyperalgesia

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Abstract

Objective Although electroacupuncture (EA) therapy is used to relieve various kinds of pain, the optimal frequency and duration of EA remain unclear. We investigated the effect of varying frequency and duration of EA during hyperalgesia elicited by carrageenan-induced inflammation.

Methods Carrageenan was administered by subcutaneous intraplantar injection to induce inflammation. Nociceptive thresholds were measured using the paw pressure threshold (PPT) (Randall-Selitto Test). EA was applied at 3, 15, or 100 Hz to the left anterior tibial muscles for 1, 15, or 60 minutes. Intensities used were chosen within the known tolerance of the animal, and increased up to 3 mA for 3 Hz, and up to 1.5 mA for 15 and 100 Hz. EA was started three hours after carrageenan injection.

Results Three hours after carrageenan injection, a marked ipsilateral inflammatory response appeared and PPT decreased significantly. This decrease persisted for at least 24 hours after carrageenan injection. EA at 3 Hz (60 minutes) resulted in significant increases of PPT which persisted for 24 hours after injection. EA at 3 Hz (15 minutes) also induced PPT elevations immediately and for one hour after EA compared to the control group. However, no other variety of EA significantly increased PPT.

Conclusion These results show that EA produces electroacupuncture analgesia of carrageenan-induced hyperalgesia. These findings also suggest that, among the frequencies and durations tested, EA at 3 Hz (60 minutes) is the most suitable frequency and duration for carrageenan-induced inflammation. It seems that EA has different analgesic effects and mechanisms according to the parameters of stimulation. For EA in the clinical induction of analgesia, it is especially important that an effective frequency and duration are selected.

Keywords Electroacupuncture, analgesia, 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 is reversed by naloxone, an opioid receptor antagonist, and that the level of β-endorphin or enkephalin in the cerebrospinal fluid increases after EA. Endogenous opioids and descending pain inhibitory systems have therefore been considered to be involved in EAA.

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. The excitability and the receptive field size of the dorsal horn neurons also increase during inflammation. It has been shown that the number of Fos-like immunoreactive neurons is significantly increased in all layers of the ipsilateral spinal cord after intraplantar (ipl) injection of carrageenan. Moreover, it has been reported that localised inflammation of a rat’s hindpaw elicits an accumulation of immune cells containing opioid peptides and that these peptides are released by

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environmental stimuli in the inflamed tissue, which may inhibit pain by interacting with peripheral opioid receptors.11

At present, EA is used clinically to relieve various kinds of pain. However, little is known about the effect of EA during inflammatory pain, and the effect of frequency and duration of EA remains unclear. Under such inflammatory conditions as mentioned above, it is predicted that distinct mechanisms are involved in EAA during inflammatory pain. Our previous study has shown that EAA in rats with inflammatory pain differs from that in normal rats. Moreover, the local immune system may also be involved in EAA in rats with inflammatory pain.12

In the present study, we investigated the effect of varying frequency and duration of EA during hyperalgesia elicited by carrageenan-induced inflammation to determine the optimal frequency and duration of EA.

**Method**

**Animals**

Male Sprague Dawley rats weighing 280-380g purchased from Japan SLC Inc were divided into the following 11 groups (n=5 per group): (1) carrageenan control (given no treatment), (2) 3Hz EA (1 minute), (3) 3Hz EA (15 minutes), (4) 3Hz EA (60 minutes), (5) 15Hz EA (1 minute), (6) 15Hz EA (15 minutes), (7) 15Hz EA (60 minutes), (8) 100Hz EA (1 minute), (9) 100Hz EA (15 minutes), (10) 100Hz EA (60 minutes), and (11) sham EA (60 minutes, needle insertion without electrical stimulation). The animals were kept at 24±1°C and a relative humidity of 50% to 60%. Standard laboratory rodent food and tap water were available ad libitum. All experiments were conducted in the light phase of a 12/12 hours (7am/pm) light/dark cycle. This study followed 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 the guidelines of our Institutional Committee on the Treatment of Experimental Animals.

**Induction of Inflammation**

Carrageenan (2%, 0.1 ml; Sigma, St Louis, MO) was administered by subcutaneous ipl injection of the left hind paw of rats under ether anaesthesia 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 paw pressure threshold (PPT), was determined. We used a cut-off threshold of 250g to avoid tissue damage to the paw.

The mean of two consecutive measurements, separated by two minutes, was determined after a rest period of 15 minutes. PPT was determined 15 minutes before, just before, and three, four, five, seven, nine, and 24 hours after the carrageenan injection.

**Electroacupuncture**

A pair of stainless steel acupuncture needles 0.20mm in diameter and 30mm in length (Seirin Corporation) was inserted into the acupoint Zusanli (ST36) and 5mm from Zusanli (the left anterior tibial muscles). One of 3, 15, or 100Hz biphasic square wave pulses of 0.1ms width were delivered via the needles for periods of 1, 15, or 60 minutes using a constant current programmed pulse generator. EA was started from three hours after carrageenan injection. The intensity of 3Hz EA was increased according to a schedule of 1-2-3mA. The intensity of 15 and 100Hz EA was increased according to a schedule of 0.5-1.0-1.5mA. Each parameter was given using an incremental change of intensity. These intensities were chosen because they are within the tolerance level of a conscious animal.

The rats were gently restrained under a soft cloth jacket during the PPT measurement and EA procedure. Except at these times, rats were left in their cage to move freely. In the control groups, the rats were gently restrained under a soft cloth jacket. The animals remained awake and still during treatment, and no signs of distress were observed.

**Data Analysis**

The experimental data are presented as means ± SD. Repeated measures ANOVA was performed to determine the overall effect. Tukey’s *post hoc* test was then used to determine probability values when repeated measures ANOVA indicated a significant effect. *P*<0.05 was considered as statistically significant.
Results

Figure 1 shows the time course of analgesia produced by one minute EA during inflammatory hyperalgesia. In the control group, PPT just before carrageenan injection was 83.9±13.4g. Three hours after the carrageenan injection, a marked ipsilateral inflammatory response (swelling and redness) appeared and PPT decreased significantly (54.1±14.2g). Moreover, this decrease continued for 24 hours after carrageenan injection. In all the EA groups, PPT also decreased three hours after the carrageenan injection. Also in all the EA groups, PPT did not increase after the EA and significant differences were not observed compared with that of the control group. In a previous study, we confirmed that little PPT elevation was observed in the control groups in which the animals were simply restrained.

Figure 2 shows the time course of analgesia produced by 15 minutes EA during inflammatory hyperalgesia. In the 3Hz EA group, PPT significantly increased four and five hours after the carrageenan injection compared with that of the control group (91.8±12.9g and 83.8±6.9g, respectively) (P<0.001, P<0.05). However, in the 15 and 100Hz EA groups, PPT did not increase after the EA compared with that of the control group.

Figure 3 shows the time course of analgesia produced by 60 minutes EA during inflammatory hyperalgesia. In all the EA groups, PPT decreased to almost the same level as the control group three hours after the carrageenan injection. However, PPT increased significantly (112.0±19.3g) immediately after the 3Hz EA (four hours after the carrageenan injection) (P<0.001). The PPT elevations produced by 3Hz EA tended to last at least 20 hours after the EA (24 hours after carrageenan injection). However, in the 15Hz and 100Hz EA groups, PPT did not increase after the EA compared with that of the control group.

Figure 4 shows the time course of analgesia produced by sham EA (60 minutes) during inflammatory hyperalgesia. Sham EA did not induce PPT elevations compared with 3Hz EA (60 minutes).

Discussion

We investigated the effect of varying frequency and duration of EA during hyperalgesia elicited by carrageenan-induced inflammation. The results showed that certain forms of EA reduced...
Figure 2 Effect of 15 minutes EA during hyperalgesia elicited by carrageenan-induced inflammation (n=5 in each group). The results are expressed as mean ± SD; *P<0.001, #P<0.05 (control versus 3Hz).

Figure 3 Effect of 60 minutes EA during hyperalgesia elicited by carrageenan-induced inflammation (n=5 in each group). The results are expressed as mean ± SD; *P<0.001 (control versus 3Hz).
carrageenan-induced hyperalgesia. In particular, the results showed that analgesia produced by 3Hz EA (15 and 60 minutes) at 3mA persisted for five and 24 hours after the carrageenan injection, respectively, and 3Hz EA (60 minutes) at 3mA induced a greater analgesia than any other frequency and duration, at the maximum tolerable intensity, as current greater than 1.0mA at 15 and 100Hz was noxious for the animals.

These results are consistent with several other animal studies of EA evoked analgesia during inflammatory pain. Zhang et al found that EA markedly reduced the numbers of carrageenan-induced Fos-like immunoreactive neurons in spinal cord lamina I–II and V–VI, and produced antinociception. In a rat model using a formalin test, Hsieh et al have shown that both 2 and 15Hz EA reduce the frequency of biting and licking and that 100Hz EA has a greater antinociceptive effect than 2 and 15Hz EA in the late phase, suggesting that different analgesic mechanisms are involved in the response to 2, 15, and 100Hz EA. In the other study, 10Hz EA also induced pre-emptive antinociception via the extra-segmental inhibition of the formalin-induced pain. In a rat model of collagen-induced arthritis, 2Hz EA produced an analgesic effect.

Many studies demonstrated that EA produced antinociception on a persistent inflammatory pain rat model, induced by injecting complete Freund’s adjuvant unilaterally into a hind paw. In a kaolin and carrageenan model of knee joint inflammation, both 4 and 100Hz TENS resulted in a reversal of hyperalgesia immediately following treatment and 100Hz TENS persisted for at least 24 hours; moreover, 4Hz TENS produces antinociception through µ-opioid receptors and 100Hz TENS that produced antinociception through δ-opioid receptors in the spinal cord. In a study that examined the effect of varying frequency, intensity, and pulse duration of TENS on carrageenan-induced hyperalgesia, although 100Hz TENS reduced hyperalgesia for up to one day after treatment, varying intensity or pulse duration had no effect on the degree of antinociception produced by 100Hz TENS. In the present study, no EA except for 3Hz EA (15 and 60 minutes) produced analgesia, and EA produced different EAA according to the frequency and duration used. Our results are also consistent

Figure 4: Effect of sham and 60 minutes EA during hyperalgesia elicited by carrageenan-induced inflammation (n=5 in each group). The results are expressed as mean ± SD; *P<0.001 (sham versus 3Hz).
with the long-lasting effect of TENS, it is considered that systemic humoral factors are involved in the EAA produced by 3Hz EA (15 and 60 minutes).

On the other hand, numerous investigations of the mechanism underlying EAA have been performed in healthy human subjects and normal animals. Low and high frequency EA-induced analgesia have been shown to be mediated by different brain substrates and different opioid peptides. Analgesia induced by low-frequency stimulation is mediated by β-endorphin and enkephalin interacting with µ- and δ-opioid receptors, while analgesia produced by high frequency stimulation is mediated through the activation of κ-opioid receptor by dynorphin. Guo et al have also reported that 2Hz EA induces much more Fos expression than 100Hz EA in the arcuate nucleus, and that 100Hz, but not 2Hz EA, induces Fos expression in the parabrachial nucleus, suggesting there are distinct neuronal pathways underlying the effect of EA at different frequencies. Moreover, 2 and 100Hz EA exert differential effects on opioid gene expression: while 2Hz EA induces a more extensive and intensive proenkephalin (PPE) mRNA expression than 100Hz EA, it has no effect on preprodynorphin (PPD) mRNA expression, which is significantly increased by 100Hz EA stimulation. In regard to the parameters of EA, Romita et al have shown that EAA differs according to the intensity, frequency, and duration of stimulation, suggesting the specific parameters of EA for the alleviation of pain are important to achieve optimal analgesia. In addition, manipulation combined with EA produces a more potent antinociception than when EA alone is applied. Thus, it is known that EA has different analgesic effects and mechanisms according to specific parameters of stimulation under normal conditions.

Our results also varied according to the specific parameters of EA during hyperalgesia elicited by carrageenan-induced inflammation. In particular, 60 minutes EA (15 and 100Hz) did not produce analgesia, even though the EA was performed for a long duration. These findings suggest the importance of frequency of EA in determining the effect of EAA and that proper administration of acupuncture is a cardinal factor in its therapeutic effects for various diseases. Also, this was in contradiction to other reports that indicated that 15 and 100Hz EA produced analgesia. Lao et al showed that 10 and 100Hz EA at an intensity of 3mA produced the greatest antinociception and twenty minutes treatment induced better antinociception than longer and shorter (10 and 30 minutes). These results also were different from ours. Although the reasons for this remain unclear in this study, it may involve differences between animal models' responses to the frequency, intensity and treatment duration of EA. We used the carrageenan-induced hyperalgesia model, while they induced complete Freund’s adjuvant model. However, our results suggested that there were distinct neuronal pathways underlying EAA produced by different frequencies and durations of EA during carrageenan-induced hyperalgesia. Zhang et al showed that 10 and 100Hz EA-induced antinociception was mediated by µ- and δ-opioid receptors in complete Freund’s adjuvant model. In contrast, it has been reported that EAA in uninjured animals is mediated by µ- and δ-opioid receptors at 2-15Hz and by κ-opioid receptors at 100Hz. We previously reported that EAA produced by 3Hz (60 minutes) under carrageenan-induced inflammation differed from that in uninjured animals, and that peripheral opioid receptors were involved in EAA under inflammatory conditions. Accordingly, it is considered that EAA in carrageenan-induced hyperalgesia differed from that in uninjured conditions.

Taken together, during carrageenan-induced hyperalgesia, 3Hz EA (15 and 60 minutes) produced long-lasting analgesic effect and 3Hz EA (60 minutes) is the most suitable frequency and duration. Thus, during inflammatory conditions as well as other conditions, EA has different analgesic effects and mechanisms according to the parameters of stimulation. For use of EA in the clinical induction of analgesia, it is especially important that an effective frequency and duration are selected.

**Summary points**

Although EA therapy is used to relieve various kinds of pain, the optimal frequency and duration of EA remain unclear.

This study showed that EA produced EAA, and 3Hz EA (60 minutes) was the most suitable frequency and duration, from those tested, for carrageenan-induced inflammation in rats.

For use of EA in the clinical induction of analgesia, it is especially important that an appropriate frequency and duration are selected.
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Reference list

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