Corticotropin-Releasing Factor
and Interleukin-1β are Involved in
the Electroacupuncture-Induced Analgesic
Effect on Inflammatory Pain Elicited
by Carrageenan

Reina Sekido,* Keisou Ishimaru† and Masakazu Sakita*

*Departments of Surgery, and †Clinical Acupuncture and Moxibustion
Graduate School of Acupuncture and Moxibustion, Meiji University of Oriental Medicine
629-0392, Japan

Abstract: Electroacupuncture (EA) is used to relieve various kinds of pain. However, the mechanistic basis of electroacupuncture analgesia (EAA) in inflammatory pain remains unclear. In the present study, we investigated whether endogenous peripheral corticotropin-releasing factor (CRF) or interleukin-1β (IL-1) participated in EAA during hyperalgesia elicited by carrageenan-induced inflammation. Carrageenan was subcutaneously administered by intraplantar (i.pl.) injection of the left hind paw to induce inflammation. Nociceptive thresholds were measured using the paw pressure threshold (PPT) (Randall Sellito Test). Rats received 3 Hz EA in the left anterior tibial muscles for 1 hour after carrageenan injection. The selective CRF antagonist, α-helical CRF, or the recombinant IL-1 receptor antagonist, IL-1ra, was administered by i.pl. injection of the inflamed paw or by intravenous (i.v.) injection 1 hour before EA. PPT decreased significantly 3 hours after carrageenan injection. This decrease persisted at least 24 hours after carrageenan injection. EA resulted in significant increases of PPT, moreover, PPT elevations lasted 24 hours after carrageenan injection. By contrast, PPT elevations produced by EA were dose-dependently antagonized by local i.pl. injection of α-helical CRF or IL-1ra. This PPT elevation was not influenced by i.v. injection of α-helical CRF or IL-1ra. These findings suggest that peripheral CRF or IL-1 participate in EAA during hyperalgesia. The release of CRF or IL-1 elicited by EA may trigger the release of opioid peptides within inflamed tissue which may activate peripheral opioid receptors and inhibit the pain.

Keywords: Electroacupuncture; Analgesia; Corticotropin-releasing Factor; IL-1β; Carrageenan; Hyperalgesia; Inflammation.
Introduction

Pain, evoked by local injury and inflammation, can be controlled by various endogenous mechanisms. Under inflammatory conditions, opioid-containing immune cells migrate preferentially to inflamed sites (Przewlocki et al., 1992; Stein, 1995; Cabot et al., 1997). Experimental and clinical studies demonstrate that locally administered opioid receptor agonists can produce pronounced analgesic effects by interacting with peripheral opioid receptors in inflamed tissue (Antonijevic et al., 1995; Stein et al., 1989, 1990a, 1993 and 1995). Stein et al. (1990a and b) have also shown that environmental stimuli, such as in the cold water swim (CWS) in rats with unilateral hind paw inflammation elicit peripheral opioid receptor-mediated anti-nociception in the inflamed paw. These opioids are released locally from immunocytes during environmental stimuli and inhibit pain in animals (Stein et al., 1990a and b) and in humans (Stein et al., 1993). Moreover, these opioid peptides can be released by stimulating immunocytes with corticotropin-releasing factor (CRF) or interleukin-1β (IL-1) in vivo and in vitro (Schafer et al., 1994; Cabot et al., 2001). It has also been shown that the anti-nociception produced by CWS is blocked by intraplantar (i.pl.) injection of CRF antagonist, suggesting a peripheral receptor-specific action of endogenous CRF (Schafer et al., 1996). Thus, it is believed that local immune system and peripheral opioid receptors are involved in inflammatory pain control (Stein et al., 1993; Stein, 1995).

At present, electroacupuncture (EA) is used to relieve various kinds of pain. However, little is known about the effects of electroacupuncture analgesia (EAA) on inflammatory pain. In a previous study, we observed that peripheral opioid receptors might be involved in the EAA in inflamed tissue (Sekido et al., 2000). In this study, we investigated whether peripheral CRF or IL-1 might be the endogenous trigger for local opioid release and was involved in EAA in 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% 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-hour (7 am/7 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 subcutaneously administered by i.pl. injection of the left hind paw of rats under ether anesthesia using a 26-gauge needle to induce inflammation.
Experimental Protocols

To determine the involvement of peripheral CRF or IL-1 on local opioid release, we examined whether α-helical CRF or recombinant IL-1 receptor antagonist (IL-1ra) given by i.pl. injection to the inflamed paw blocked the paw pressure threshold (PPT) elevations produced by EA. Animals received i.pl. injection (0.1 ml) of α-helical CRF (2.5, 3.7 and 5.0 ng) or IL-1ra (25, 50 and 100 ng) 1 hour before EA (n = 6 per group). To confirm that these effects were not mediated through a central site of action, another group of animals received an intravenous (i.v.) injection (0.1 ml) of 5.0 ng α-helical CRF or 100 ng IL-1ra 1 hour before EA (n = 6 per group).

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 onto the dorsal surface of the left hind paw. The pressure required to elicit paw withdrawal, the PPT, was determined. We used a cut-off threshold of 250 g to avoid tissue damage to the paw. 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.

Electroacupuncture

A pair of stainless steel needles, 0.20 mm in diameter and 30 mm in length, were inserted into an acupoint of Zusanli (ST36) and the left anterior tibial muscles (5 mm from the Zusanli). A 3 Hz biphasic square wave pulse of 0.1 ms pulse width was delivered via the needles for a period of 60 minutes using a constant current programmed pulse generator. EA was started from 3 hours after carageenan injection. 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 the PPT measurement and EA procedure. Except at these times, rats were left in their cage to move freely.

Drugs

We used the selective CRF antagonist α-helical CRF (Sigma) and recombinant IL-1 receptor antagonist IL-1ra (R&D Systems). Both agents were dissolved in sterile distilled water and administered by i.pl. injection (0.1 ml) of the inflamed paw or i.v. injection via a tail vein 1 hour before EA. Control animals received 0.1 ml of sterile distilled water.
Data Analysis

The experimental data were expressed as means ± SD. Repeated measures ANOVA were performed to determine the overall effect. Tukey’s post-hoc test was then used to determine probability values when repeated measure ANOVAs indicated a significant effect. p < 0.05 was considered as statistically significant.

Results

Effect of EA during Hyperalgesia Elicited by Carrageenan-induced Inflammation

Figure 1 shows the time course of analgesia produced by EA during inflammatory hyperalgesia. In the control group, PPT just before carrageenan injection was 83.9 ± 13.4 g. Three hours after the carrageenan injection, a marked ipsilateral inflammatory response (swelling and redness) appeared and PPT decreased significantly (54.1 ± 14.2 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 (86.4 ± 12.3 g) immediately after the EA (p < 0.001). The PPT elevations produced by EA lasted at least 20 hours after the EA (24 hour carrageenan injection) (Fig. 1).

Figure 1. Effects of EA during hyperalgesia elicited by carrageenan-induced inflammation. The results are expressed as mean ± SD. n = 6 per group, *p < 0.001, †p < 0.01, ‡p < 0.05 (control versus EA).
Effect of i.pl. Injection of α-Helical CRF on EAA during Hyperalgesia Elicited by Carrageenan-induced Inflammation

Figure 2A shows the effect of i.pl. injection of α-helical CRF on EAA during inflammatory hyperalgesia. To observe the effect of α-helical CRF on the PPT elevations induced by EA, rats were given an i.pl. injection of α-helical CRF (2.5, 3.7 and 5.0 ng) or vehicle 1 hour before EA. In the α-helical CRF (2.5, 3.7 and 5.0 ng) + EA or vehicle + EA groups, PPT decreased to almost the same level as the control group 3 hours after the carrageenan injection. In the vehicle + EA group, PPT significantly increased immediately, 5 hours and 20 hours after termination of EA (89.1 ± 10.5 g, 77.7 ± 10.4 g and 78.5 ± 6.4 g, respectively), compared to that of the control group. In the 2.5 ng α-helical CRF + EA group, PPT increased to the same level as the vehicle + EA group after the termination of EA, and no differences were observed between these two groups. In the 3.7 ng α-helical CRF + EA group, PPT tended to increase immediately after the termination of EA and thereafter compared to that of the control group. In the 5.0 ng α-helical CRF + EA group, however, PPT did not increase immediately, 5 hours and 20 hours after the termination of EA (55 ± 6.1 g, 53.7 ± 2.6 g and 48.7 ± 4.1 g, respectively) and no differences were observed between this group and the control group, but significant differences were observed compared to that of the vehicle + EA group.

PPT elevations produced by EA were not influenced by i.v. injection of 5.0 ng α-helical CRF and PPT significantly increased during 24 hours after the carrageenan injection compared to that of the control group (Fig. 2B).

Effect of i.pl. Injection of IL-1ra on EAA during Hyperalgesia Elicited by Carrageenan-induced Inflammation

Figure 3A shows the effect of i.pl. injection of IL-1ra on EAA during inflammatory hyperalgesia. Rats were given an i.pl. injection of 25, 50 and 100 ng IL-1ra 1 hour before EA. In the IL-1ra (25, 50 and 100 ng) + EA groups, PPT decreased to the same level as the control group 3 hours after carrageenan injection. In the 25 ng IL-1ra + EA group, PPT increased to the same level as the vehicle + EA group immediately, 1 hour and 5 hours after the termination of EA (86.8 ± 13.3 g, 81.0 ± 7.9 g and 80.4 ± 9.6 g, respectively). In the 50 ng IL-1ra + EA group, PPT tended to increase after the termination of EA compared to that of the control group. In the 100 ng IL-1ra + EA group, however, PPT did not increase after the termination of EA.

Thus, PPT elevations elicited by EA were dose-dependently antagonized by i.pl., but not by i.v. injection of α-helical CRF or IL-1ra (Figs. 2 and 3). In addition, PPT elevations elicited by EA were not antagonized by i.v. injection of 100 ng IL-1ra (Fig. 3B). Moreover, neither i.pl. injection of α-helical CRF nor IL-1ra per se did not have analgesic or hyperalgesic effects during carrageenan-induced inflammation (Table 1).
Figure 2. Effects of i.pl. (A) or i.v. (B) injection of α-helical CRF on EAA during hyperalgesia elicited by carrageenan-induced inflammation. The results are expressed as mean ± SD. n = 6 per group, *p < 0.001, †p < 0.01, ††p < 0.05 (control versus vehicle + EA, vehicle + EA versus 2.5 ng + EA, vehicle + EA versus 5.0 ng + EA, control versus 5.0 ng i.v. + EA).
Figure 3. Effects of i.pl. (A) or i.v. (B) injection of IL-1ra on EAA during hyperalgesia elicited by carrageenan-induced inflammation. The results are expressed as mean ± SD. n = 6 per group. *p < 0.001, #p < 0.01, †p < 0.05 (control versus vehicle + EA, vehicle + EA versus 25 ng + EA, vehicle + EA versus 100 ng + EA, vehicle + EA versus 50 ng + EA, control versus 100 ng i.v. + EA).
Table 1. Effects of i.pl. Injection of α-helical CRF or IL-1ra During Hyperalgesia Elicited by Carrageenan-induced Inflammation

<table>
<thead>
<tr>
<th>Group</th>
<th>-15 Minutes</th>
<th>0 Hours</th>
<th>3 Hours</th>
<th>4 Hours</th>
<th>5 Hours</th>
<th>7 Hours</th>
<th>9 Hours</th>
<th>24 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrageenan control (n = 6)</td>
<td>86.2 ± 15.3</td>
<td>83.9 ± 13.4</td>
<td>54.1 ± 14.2</td>
<td>54.1 ± 9.2</td>
<td>60.0 ± 16.6</td>
<td>58.7 ± 20.3</td>
<td>56.4 ± 9.4</td>
<td>57.5 ± 8.2</td>
</tr>
<tr>
<td>Carrageenan + α-helical CRF 2.5 ng (n = 3)</td>
<td>73.3 ± 11.6</td>
<td>72.0 ± 10.1</td>
<td>58.7 ± 11.4</td>
<td>43.7 ± 9.7</td>
<td>43.7 ± 5.0</td>
<td>49.5 ± 6.8</td>
<td>47.9 ± 10.6</td>
<td>50.8 ± 7.3</td>
</tr>
<tr>
<td>Carrageenan + α-helical CRF 3.7 ng (n = 3)</td>
<td>74.5 ± 8.7</td>
<td>72.0 ± 10.0</td>
<td>55.0 ± 4.5</td>
<td>53.3 ± 4.3</td>
<td>53.3 ± 6.2</td>
<td>50.4 ± 5.7</td>
<td>50.8 ± 5.0</td>
<td>58.3 ± 8.5</td>
</tr>
<tr>
<td>Carrageenan + α-helical CRF 5.0 ng (n = 3)</td>
<td>77.5 ± 19.2</td>
<td>77.9 ± 22.5</td>
<td>53.7 ± 9.9</td>
<td>51.2 ± 8.7</td>
<td>50.8 ± 8.3</td>
<td>48.7 ± 3.3</td>
<td>55.0 ± 2.5</td>
<td>60.4 ± 4.7</td>
</tr>
<tr>
<td>Carrageenan + IL-1ra 25 ng (n = 3)</td>
<td>65.4 ± 4.0</td>
<td>64.1 ± 1.9</td>
<td>46.6 ± 8.1</td>
<td>45.4 ± 0.7</td>
<td>42.5 ± 1.2</td>
<td>45.8 ± 8.0</td>
<td>48.3 ± 5.0</td>
<td>51.6 ± 5.2</td>
</tr>
<tr>
<td>Carrageenan + IL-1ra 50 ng (n = 4)</td>
<td>78.7 ± 9.1</td>
<td>75.9 ± 6.8</td>
<td>55.9 ± 2.5</td>
<td>52.1 ± 4.8</td>
<td>46.8 ± 5.9</td>
<td>53.7 ± 6.5</td>
<td>52.1 ± 4.6</td>
<td>55.6 ± 5.2</td>
</tr>
<tr>
<td>Carrageenan + IL-1ra 100 ng (n = 4)</td>
<td>75.9 ± 15.8</td>
<td>78.4 ± 18.7</td>
<td>57.8 ± 12.1</td>
<td>57.1 ± 11.1</td>
<td>50.0 ± 5.3</td>
<td>57.0 ± 16.7</td>
<td>55.3 ± 9.8</td>
<td>63.1 ± 10.2</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD (g) and as percentage change from PPT of 15 minutes before carrageenan injection (100%). n = 6; control, n = 3–4 per group.
Discussion

EA has been widely used to relieve various kinds of pain. Numerous investigations of the mechanism underlying EAA have been performed in humans and animals. The results have shown 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 was increased after EA (Clement-Jones et al., 1979 and 1980; He, 1987). Although it is well known that EAA is at least partially mediated by endogenous opioids and other neurotransmitters in the central nervous system (CNS) (Han and Trenius, 1982; He, 1987), the mechanism underlying EAA during inflammatory pain is unclear. In a previous study, we showed that the EAA during inflammatory hyperalgesia lasted longer and was blocked by i.pl. injection of naloxone (Sekido et al., 2000). This indicated that peripheral opioid receptors might be involved in the EAA during inflammatory hyperalgesia. We then investigated whether the endogenous peripheral CRF or IL-1 participated in EAA during hyperalgesia elicited by carrageenan-induced inflammation.

In the present study, we found that the EAA in inflamed tissue could be dose-dependently blocked by local i.pl. injection of α-helical CRF or IL-1ra. This phenomenon is in accordance with the finding that anti-nociception elicited by CWS is blocked by i.pl. injection of α-helical CRF (Schafer et al., 1996).

CRF and IL-1 receptors have been demonstrated to be expressed in splenocytes, macrophages, and T- and B-lymphocytes (Webster et al., 1990; Dinarello and Thompson, 1991; Karalis et al., 1991; Crofford et al., 1992; Mousa et al., 1996) and these receptors are upregulated in inflamed tissue (Mousa et al., 1996). CRF and IL-1 receptors are also detectable in the CNS (Heijnen et al., 1991). However, peripheral CRF and IL-1 receptors on immunocytes were probably involved in the EAA since the EAA in inflamed tissue could not be blocked by i.v. injection of α-helical CRF or IL-1ra.

There are several differences between CWS-induced anti-nociception and EAA. First, EAA in inflamed tissue lasted 20 hours after termination of EA while the CWS-induced anti-nociception returned to baseline 10–17 minutes after termination of CWS (Schafer et al., 1996). Secondly, EAA was blocked by i.pl. injection of both α-helical CRF or IL-1ra. However, CWS-induced anti-nociception could not be blocked by i.pl. injection of IL-1ra (Schafer et al., 1996). These differences might be due to the difference in inflammatory agent used and stage of inflammatory reaction. Stein et al. used Freund’s complete adjuvant to induce inflammation and CWS was conducted 4 days after inoculation. By contrast, we used carrageenan to induce inflammation and EA was conducted 3 hours after carrageenan injection. Antonijevic et al. (1995) showed that inflammation-induced disruption of the perineurial barrier and peripheral opioid analgesia coincides with earlier stages of an inflammatory reaction. In this study, disruption of the perineurial barrier might be involved in EAA. IL-1 gene expression has also been shown to peak very early, i.e. 1–2 hours after inflammatory stimuli (Ulich et al., 1992). Therefore, the EAA might be antagonized by i.pl. injection of IL-1ra.

The other possible difference between EAA and CWS-induced anti-nociception is in the mechanism of action. It is well known that the analgesic effect of EA is mediated by
endogenous opioids and other neurotransmitters including serotonin and norepinephrine
in the CNS (Han and Trenius, 1982). Therefore, we could not completely exclude the
involvement of the CNS in the mechanism of EAA in rats with carrageenan-induced
inflammation.

Taken together, during hyperalgesia elicited by carrageenan-induced inflammation, EA
might be able to release CRF and IL-1 from immunocytes within inflamed tissue. CRF or
IL-1 triggers the release of opioid peptides within inflamed tissue and these peptides activate
peripheral opioid receptors, reducing neuronal excitability or release of proinflammatory
neuropeptides (e.g. substance P), thereby inhibiting pain. Thus, in addition to the endogenous
opioid mechanism in the CNS, the local immune system may be involved in the EAA. Further
study is needed to clarify the mechanism of EAA during peripheral inflammation.

References
Antonijevic, I., S.A. Mousa, M. Schäfer and C. Stein. Perineurial defect and peripheral opioid analgesia
Cabot, P.J., L. Carter, C. Gaiddon, Q. Zhang, M. Schäfer, J.P. Loeffler and C. Stein. Immune cell-
Chen, X.H., E.B. Geller and M.W. Adler. Electrical stimulation at traditional acupuncture sites in
in heroin addicts: changes in met-enkephalin and beta-endorphin in blood and CSF. Lancet ii:
Clement-Jones, V., L. McLoughlin, S. Tomlin, G.M. Besser, L.H. Rees and H.L. Wen. Increased beta-
endorphin but not met-enkephalin levels in human CSF after acupuncture for recurrent pain.
Local secretion of corticotropin-releasing hormone in the joints of Lewis rats with inflammatory
Dinarello, C.A. and R.C. Thompson. Blocking IL-1: interleukin-1 receptor antagonist in vivo and
Karalis, K., H. Sano, J. Redwine, S. Listwak, R.L. Wilder and G.P. Chrousos. Autocrine or paracrine
Mayer, D.J., D.D. Price and A. Rafii. Antagonism of acupuncture analgesia in man by the narcotic


