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Electroacupuncture-induced analgesia in a rat model of ankle sprain pain is mediated by spinal α-adrenoceptors

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Abstract

In a previous study, we showed that electroacupuncture (EA) applied to the SI-6 point on the contralateral forelimb produces long-lasting and powerful analgesia in pain caused by ankle sprain in a rat model. To investigate the underlying mechanism of EA analgesia, the present study tested the effects of various antagonists on known endogenous analgesic systems in this model. Ankle sprain was induced in anesthetized rats by overextending their right ankle with repeated forceful plantar flexion and inversion of the foot. When rats developed pain behaviors (a reduction in weight-bearing of the affected hind limb), EA was applied to the SI-6 point on the contralateral forelimb for 30 min under halothane anesthesia. EA significantly improved the weight-bearing capacity of the affected hind limb for 2 h, suggesting an analgesic effect. The α-adrenoceptor antagonist phentolamine (2 mg/kg, i.p. or 30 μg, i.t.) completely blocked the EA-induced analgesia, whereas naloxone (1 mg/kg, i.p.) failed to block the effect. These results suggest that EA-induced analgesia is mediated by α-adrenoceptor mechanisms. Further experiments showed that intrathecal administration of yohimbine, an α_2-adrenergic antagonist, reduced the EA-induced analgesia in a dose-dependent manner, whereas terazosin, an α_1-adrenergic antagonist, did not produce any effect. These data suggest that the analgesic effect of EA in ankle sprain pain is, at least in part, mediated by spinal α_2-adrenoceptor mechanisms.

Keywords: Descending inhibitory system; Electroacupuncture; Naloxone; Phentolamine; Weight-bearing force; Noradrenergic inhibitory system

1. Introduction

The endogenous opioid system has received much attention as the key underlying mechanism of acupuncture analgesia (Han and Terenius, 1982; He, 1987; Han, 1993; Mayer, 2000), ever since it was first demonstrated that the analgesic effect of acupuncture could be reversed by naloxone, an opioid antagonist (Pomeranz and Chiu, 1976; Mayer et al., 1977). However, under certain circumstances, opioid antagonists have failed to reverse acupuncture-induced effects in rabbits (McLennan et al., 1977), in rats (Das et al., 1984; Bossut et al., 1991; Kwon et al., 2001; Koo et al., 2002), and in humans (Chapman et al., 1980, 1983). Therefore, the mechanism of acupuncture analgesia is still unclear. The contradictory findings on the actions of naloxone on the effects of acupuncture suggest that multiple biological mechanisms are involved and that different conditions may trigger different mechanisms. In fact, besides endogenous opioids, monoaminergic neurotransmitters may play an additional role in acupuncture

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analgesia (Cheng and Pomeranz, 1981; Han and Terenius, 1982; Takeshige et al., 1992; Mayer, 2000).

Our previous study showed that electroacupuncture (EA) produces a powerful analgesic effect in a rat model of ankle sprain pain. It is unlikely that the analgesic effect on ankle sprain pain was mediated by the endogenous opioid system because, in a previous study, systemic injection of opioid antagonists failed to block EA-induced analgesia (Koo et al., 2002). On the other hand, the monoaminergic system is a well-known pain modulation system (Millan, 2002) and monoaminergic antagonists are shown to attenuate EA-induced antinociceptive effects in studies of acute-evoked pain (Takeshige et al., 1980, 1992; Cheng and Pomeranz, 1981). In addition, it has been reported that synthesis and release of serotonin (5-HT) and norepinephrine in the central nervous system are accelerated by manual or electronic acupuncture (Han, 1986). Therefore, the present study was designed to test whether or not the monoaminergic system is involved in EA analgesia in a rat model of ankle sprain pain and if so, to identify which types of monoaminergic receptors are involved.

2. Materials and methods

2.1. Experimental animals

Experiments were performed on young adult male Sprague–Dawley rats (200–320 g, Harlan Sprague–Dawley, Indianapolis, IN, USA). Animals were housed in groups of two in plastic cages with soft bedding and were provided free access to food and water under a 12/12 h reversed light–dark cycle (dark cycle: 8:00 A.M.–8:00 P.M.). All animals were acclimated for 7 days before the experiment began. Animal experiments were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch.

2.2. Procedure for ankle sprain

The rats were placed under general anesthesia and the right ankle was sprained by manually overextending the lateral ligaments without breaking them to imitate a lateral ankle sprain in a human being (Cotler, 1984). Details of the ankle sprain procedure are described in our earlier study (Koo et al., 2002). In brief, under halothane anesthesia (in the flow of oxygen; 3% for induction and 1.5% for maintenance), the right-hind foot of each rat was repeatedly bent in the direction of simultaneous inversion and plantar flexion 60 times during a 1-min period with gradually increasing force so that the foot could eventually be bent to a position of 90° inversion and 90° plantar flexion from the resting position. The foot was then further inverted repeatedly 60 times during the next 1-min period so that it eventually reached a 180° inversion (the paw facing completely upward). These two 1-min procedures were repeated so that the total procedure took 4 min. Anesthesia was discontinued, and the rats recovered from anesthesia within 5–10 min.

2.3. Intrathecal catheter implantation

Intrathecal catheters were implanted in the rats under halothane anesthesia. The back of the rat was shaved and wiped with povidone–iodine (10%). The lamina and articular process of the T12 vertebrae were carefully removed using a rongeur, and the dura mater was exposed. A pinhole was made in the dura using a pair of microscissors, and a PE-10 catheter (Becton Dickinson, Sparks, MD, USA) was inserted into the spinal subarachnoid space between T12 and T13. The tip of the catheter was aimed at the lumbar enlargement (about 1 cm caudal to the insertion point). The tubing was secured to the muscles at multiple sites and fed subcutaneously to the mid-thoracic level in order to expose the tip at the dorsal midline position. The tip of the tubing was sealed, and the incision was closed. After full recovery from anesthesia, rats were returned to their cages and housed individually. After one week of recovery from catheter implantation, the rats underwent the ankle sprain procedure described above. At the end of each experiment, the area of catheter implantation was re-exposed, and the status of the tubing was examined. An injection of 5 μl of Evans blue dye (0.5%) showed that the tubing permitted free passage of injected material in all cases.

2.4. Drug treatment

The effect of drug treatment was examined by comparing the effects of two drugs (either two different compounds or one compound and saline control) in a cross-over design by administering two compounds in the same rats on two consecutive days (1 and 2 days post ankle sprain) in a random sequence (see Section 3 for detailed procedure).

The effect of two different drugs on EA-induced analgesia was tested by systemic intraperitoneal injection. The drugs tested included naloxone hydrochloride (1 mg/kg; Endo, Chadds Ford, PA, USA) and phentolamine mesylate (2 mg/kg; RBI, Natick, MA, USA). For the intrathecal injections, phentolamine mesylate (30 μg, RBI, Natick, MA, USA), idazoxan hydrochloride (30 μg, Sigma, St. Louis, MO, USA), terazosin hydrochloride (10 μg, Abbott Laboratories, North Chicago, IL, USA), or yohimbine hydrochloride (10 μg, Sigma, St. Louis, MO, USA) in 10 μl of saline was given immediately after termination of EA stimulation. The control group received 10 μl of saline alone. After each intrathecal injection, the catheter was flushed with 10 μl of saline.

2.5. Electroacupuncture (EA)

After placing the rats under gaseous anesthesia (halothane 1.0% in air), EA was applied by electrically stimulating an acupuncture point with a pair of bipolar stimulating electrodes, which were modified acupuncture needles. Two stainless steel acupuncture needles (0.3 mm in diameter and 30 mm in length) were mounted on a holder with 1 mm separation between the tips. The needle set was inserted into a specified acupoint at a depth of 5 mm, and electrical stimulation was applied by a

Grass S88 stimulator equipped with a SIU5 isolation unit (Grass Medical Instruments, Quincy, MA, USA). Trains of four pulses (1 ms long square wave pulses, 100 Hz of intra-train frequency), repeated at a rate of 2 Hz, were delivered at an intensity of 10 times the muscle twitch threshold (the muscle twitch threshold was about 200 μA). The current delivered was monitored at all times, and the polarity was reversed every 60 s to prevent polarization of the electrodes. The total duration of EA stimulation was 30 min. Immediately after the termination of EA, anesthesia was discontinued, and the rats usually resumed full activity within 5–10 min. Details of the EA procedure are described elsewhere (Koo et al., 2002).

EA was applied to two different sites on the forelimb contralateral to the side of the sprained ankle (Fig. 1). These two sites are equivalent to known human acupuncture points: the SI-6 ('Yangno') and LI-4 ('Hapkok') which are terms designated by the World Health Organization (1993). The SI-6 point is located at the posterior distal end of the forearm between the radius and ulna and the LI-4 point is located in the triangular space between the thumb and the index finger. These two points are about 10 mm apart in the rat. Fig. 1 shows the general layout of the locations in which EA was applied in this study.

2.6. Behavioral tests

All behavioral tests were performed by experimenters who were blinded to the exact experimental procedure. However, experiments with intrathecal injection were done in a non-blinded fashion.

To estimate the level of pain in the sprained ankle, we measured the weight-bearing force (WBF) of the affected foot. In our previous study, we found that the reduction in WBF of the foot after ankle sprain (limping) was likely due to pain. Furthermore, an improvement in WBF can be interpreted as a sign of analgesia because systemic injection of morphine restores weight bearing after ankle sprain in a dose-dependent manner (Koo et al., 2002).

Each rat was allowed to walk through a long, narrow plastic chamber (10 cm width, 10 cm high, and 60 cm long). An electronic balance (Acculab, Pocket Pro 250-B, Newton, PA, USA) was placed on the floor at the midway point of the walking path. The rectangular plate of the balance was placed so as to cover half the width of the walking path so that only the limbs on one side would step on the balance. The analog signal of the balance was fed into a digital oscilloscope (Nicolet model 410, Madison, WI, USA). The magnitude of the signal representing the WBF was used for further data analysis (as described in Section 3).

2.7. Data analysis

Data are expressed as means ± standard error of the mean (SEM). Statistical tests were conducted using two-way repeated-measures analysis of variance (ANOVA) followed by all pair-wise multiple comparison procedures (Tukey test) or by the cross-over repeated ANOVA followed by between group comparison with the least square method (Senn, 1993; Kirk, 1995; Davis, 2002), using the SAS statistical package (SAS, version 9.1). For cross-over designed experiments, carry-over effects were assumed to be negligible since the baseline values of the first and second treatment groups were essentially identical and had minimal variability. A p value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. EA-induced analgesia on ankle sprain pain

The weight-bearing force (WBF) of the right-hind limb was measured before and after ankle sprain, and also after EA application. As shown in Fig. 2, the WBF of the hind limb was approximately 60% of total body weight in normal rats (Fig. 2A and D) and then decreased to approximately 30% one day after ankle sprain (Fig. 2B and E). Our previous study (Koo et al., 2002) documented that the decrease in WBF was due to limping to favor the limb associated with pain in the sprained ankle. One hour after EA application to the SI-6 point of the contralateral forelimb, the WBF of the ankle-sprained hind limb recovered to approximately 45% of body weight (Fig. 2C). Such recovery of WBF is interpreted as a sign of analgesic effect.

Although EA applied to the SI-6 point produced a partial recovery of WBF, identical EA applied to a neighboring acupuncture points (LI-4, about 10 mm away) failed to demonstrate any improvement in WBF (Fig. 2F), suggesting that EA effect is site specific.

Most of the experiments in this study were conducted in a 2-day span after ankle sprain (24 and 48 h post-sprain). Because pre-EA baseline values were different between 1 and 2 post-sprain days due to gradual spontaneous recovery, the baseline value of WBF was

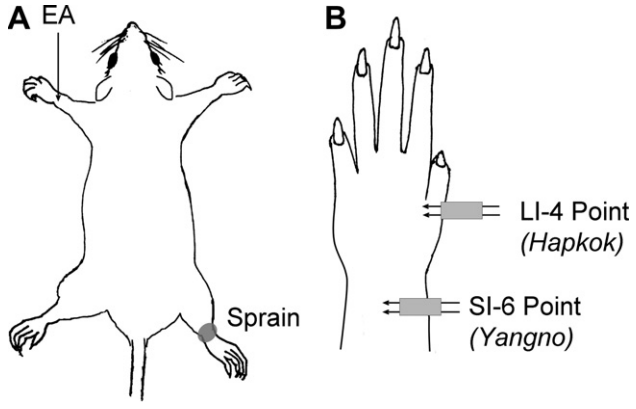

Fig. 1. Schematic drawings showing the locations of sprained ankle and electroacupuncture (EA). (A) The sprain was produced on the right ankle, and EA was applied to the acupuncture points on the left forelimb. (B) A detailed view of the left forelimb showing the locations of EA application. EA was applied with a stimulation needle set consisting of two acupuncture needles separated by 1 mm, and electrical current was applied between the two needles. The SI-6 point was used as the main point, and the LI-4 point was used as a control (sham stimulation) point.

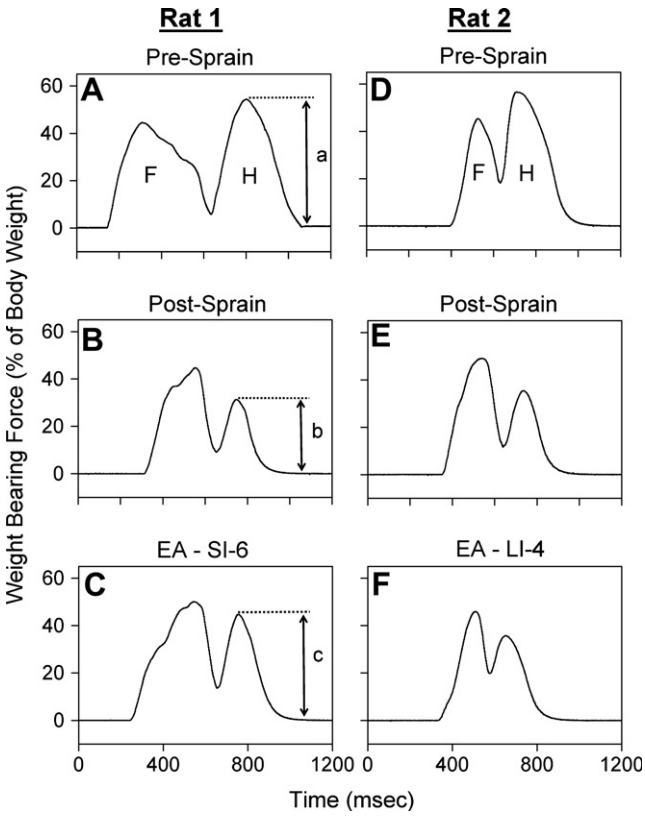

Fig. 2. Effects of EA in rats with sprained ankles. Graphs show analog output for the WBF of the forelimb (F) and hind limb (H) on one side (the right side) of two rats (A–C for rat #1 and D–F for rat #2) obtained during walking. WBF is expressed as a percentage of each rat's body weight. In normal conditions (A and D), the hind limb bore about 50–60% of the body weight (height labeled as "*a*"). One day after ankle sprain (B and E), the WBF of the hind limb with the sprained ankle was reduced to about half (height labeled as "*b*"). One hour after a 30-min application of EA to the SI-6 point, WBF significantly recovered in rat #1 (C, height labeled as "*c*"), whereas the same EA applied to the LI-4 point had no effect in rat #2 (F). For group data treatment, percentage maximum recovery after EA was calculated as follows using the height values labeled as "*a*–*c*" in (A–C): percentage of maximum recovery $= ([c-b]/[a-b]) \times 100$. Therefore, recovery is 100% (full) when the height of *c* reaches that of *a*, and is 0% (no recovery) when the height of *c* remains equal to that of *b*.

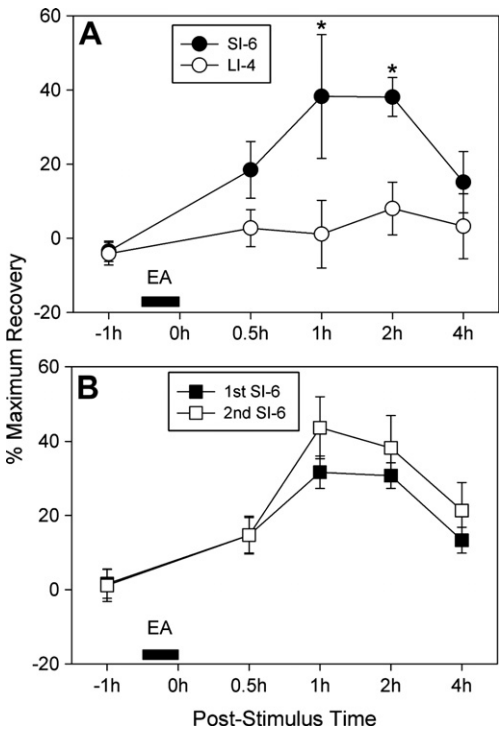

Fig. 3. Group data on EA-induced analgesia on ankle sprain pain expressed as percentage of maximum recovery. Graphs are plotted for the average values (±SEM) of percentage of maximum recovery value (100% means full recovery of WBF to pre-ankle sprain levels). (A) Comparison of effects of EA applied to the SI-6 and LI-4 points in two groups of rats (10 rats in each group). EA was applied 1 day after ankle sprain. EA to the SI-6 point (for 30 min) produced significant recovery of WBF to about 40% of full recovery lasting about 2 h. On the other hand, identical EA applied to the LI-4 point produced no recovery of WBF. An asterisk (*) indicates a value significantly ($p < 0.05$) different from the equivalent value with LI-4 stimulation by the two-way repeated measures ANOVA (time factor repetition) followed by Tukey multiple comparisons. (B) Comparison of effects of EA applied to the SI-6 points repeated on two consecutive days (1st and 2nd post-sprain days) in a group of seven rats. Two EA applications produced a comparable recovery of WBF (group factor with two-way repeated-measures ANOVA [two-factor repetition]: $F = 3.062$, $p = 0.131$).

measured before each experiment, and data were expressed as the percentage of maximum recovery. To show how the percentage of maximum recovery was calculated, the magnitudes of hind limb WBF in different conditions are labeled as *a* (normal), *b* (after ankle sprain), and *c* (after a treatment) in Fig. 2A–C. Percentage of maximum recovery $= ([c-b]/[a-b]) \times 100$. Therefore, recovery is 100% (full) when the magnitude of *c* reaches that of *a*, and it is 0% (no recovery) when the magnitude of *c* remains the same as that of *b*.

Fig. 3A shows group data on EA-induced analgesia on ankle sprain pain expressed as percentage of maximum recovery. As shown in Fig. 3A, EA applied to the SI-6 point in 10 rats with a sprained ankle (1 day post-sprain) produced significant recovery of WBF to about 40% of full recovery lasting about 2 h. On the other hand, identical EA applied to the LI-4 point in another 10 rats with a sprained ankle produced no recovery of WBF (group factor with two-way repeated-measures ANOVA [time factor repetition]: $F = 11.702$, $p = 0.003$).

Because most experiments were conducted within a 2-day span after ankle sprain and the data collected in those 2 days were pooled, it was necessary to test whether EA produced the same effect in those 2 days. In seven rats with a sprained ankle, EA applications to the SI-6 point were repeated on the first and second post-sprain days. As shown in Fig. 3B, those two EA applications produced a comparable recovery of WBF (group factor with two-way repeated-measures ANOVA

[two-factor repetition]: $F = 3.062$, $p = 0.131$), suggesting that pooling data from two post-sprain days is justified.

3.2. Effect of intraperitoneal injection of an adrenergic antagonist on EA-induced analgesia

We first examined the systemic effect of an adrenergic antagonist on EA-induced analgesia. Experiments were conducted using the cross-over design with injections of compounds in a 2-day period after ankle sprain. One day after ankle sprain, eight rats were divided randomly into two groups. In one group of four rats, phentolamine (2 mg/kg) was administered intraperitoneally immediately after the termination of EA, which was applied to the contralateral SI-6 point for 30 min. In the other group of four rats, saline vehicle was injected immediately after the termination of EA applied to the same point. The WBF of the affected hind limb was measured at 0.5, 1, 2, and 4 h after phentolamine or saline treatment. On the following day (the second post-sprain day), the procedures for these two groups were reversed. Therefore, all eight rats received phentolamine and saline injection in a 2-day period in a random sequence. Data on WBF were expressed as the percentage of maximum recovery. Fig. 4A shows group data of the eight rats. The data were analyzed using a cross-over repeated ANOVA model and fixed factors, 'injection sequence', 'post-injection time', and 'treatment group', were fitted to the data with the correlated error term. The analysis showed that the treatment factor for the two groups was significantly different ($F = 8.18$, $p = 0.0095$), whereas the factor for the injection sequence was not ($F = 0.74$, $p = 0.398$). Although the saline group showed a long-lasting recovery in WBF following EA treatment, the phentolamine group failed to show any improvement after EA, indicating that the analgesic effect of EA was blocked by systemic injection of phentolamine, an α-adrenergic antagonist. These data suggest that the adrenergic system is involved in mediating EA-induced analgesia in ankle sprain pain.

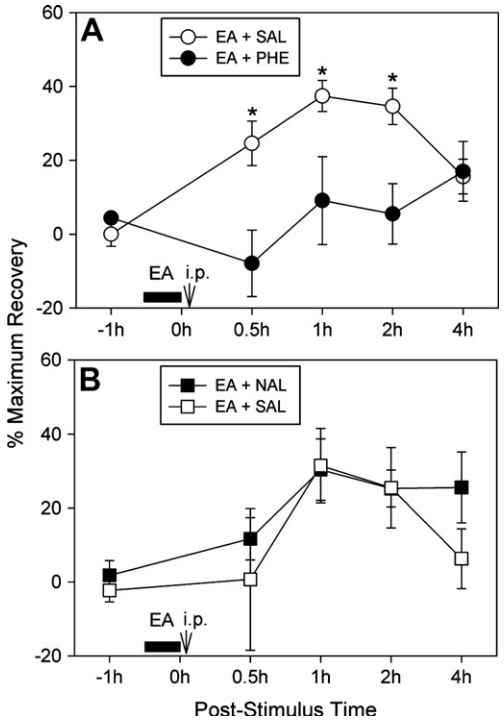

Fig. 4. Effects of systemic application of α-adrenergic and opioid receptor antagonists on EA-induced analgesia in rats with sprained ankles. Graphs are plotted for the average values (±SEM) of percentage of maximum recovery. (A) Effects of an α-adrenergic receptor antagonist on EA-induced analgesia ($n = 8$ rats). Phentolamine (2 mg/kg in 0.2 ml saline) or the same volume of saline was given intraperitoneally immediately after termination of EA (applied to the SI-6 point on the contralateral forelimb for 30 min). The experiment was done with the cross-over design so that all eight rats received both phentolamine and saline alone within a 2-day span (1st and 2nd post-sprain days). There was no significant improvement in WBF in the phentolamine-injected group, whereas the saline injection group showed a long-lasting recovery of WBF. An asterisk (*) indicates a value significantly ($p < 0.05$) different from the equivalent value after phentolamine injection, using cross-over repeated ANOVA with the least square method. (B) Effects of an opioid antagonist on EA-induced analgesia in rats with sprained ankles ($n = 7$ rats). Either naloxone (1 mg/kg in 0.2 ml of saline) or the same volume of saline was given intraperitoneally immediately after termination of EA (applied to the SI-6 point on the contralateral forelimb for 30 min). The experiment was done with the cross-over design so that all seven rats received both naloxone and saline in the 2-day span (1st and 2nd post-sprain days). Both the saline and naloxone group showed a long-lasting recovery of WBF following EA treatment (group factor with cross-over repeated ANOVA: $F = 0.62$, $p = 0.443$).

3.3. Effect of intraperitoneal injection of an opioid antagonist on EA-induced analgesia

For the next experiment, we examined the possible involvement of the endogenous opioid system in EA-induced analgesia in ankle sprain pain. Experiments were again conducted in cross-over design with injections of compounds during the first 2 days after ankle sprain. One day after ankle sprain, seven rats were divided randomly into two groups. In one group of four rats, naloxone (1 mg/kg) was given intraperitoneally immediately after the termination of EA, which was applied to the contralateral SI-6 point for 30 min. In the other group of three rats, saline vehicle was injected immediately after the termination of EA applied to the same point. The WBF of the affected hind limb was measured at 0.5, 1, 2, and 4 h after naloxone or saline treatment. The next day (2nd post-sprain day), the procedures for these two groups were reversed. Therefore, all seven rats received naloxone and saline injection in a 2-day period in random sequence. Data on WBF were expressed as the percentage of maximum recovery. Fig. 4B shows group data of the seven rats. The data were analyzed using a cross-over repeated ANOVA model and fixed factors, 'injection sequence', 'post-injection

time', and 'treatment group', fitted to the data with the correlated error term. The analysis showed that neither the treatment group factor ($F = 0.62$, $p = 0.443$) nor the sequence factor ($F = 0.07$, $p = 0.788$) was significantly different. Both the saline and naloxone groups showed a long-lasting recovery of WBF following EA treatment. These data suggest that the endogenous opioid system is not involved in mediating EA-induced analgesia in ankle sprain pain.

3.4. Effect of intrathecal injection of an adrenergic antagonist on EA-induced analgesia

Because the adrenergic system seems to be involved in mediating EA-induced analgesia, the possible involvement of the adrenergic system in the spinal cord was examined by repeating the experiment with intrathecal administration of phentolamine. Intrathecal catheters were implanted in seven rats. One week later, the ankles of these rats were sprained. Experiments were again conducted in cross-over design with injections of compounds in a 2-day period after ankle sprain. One day after ankle sprain, seven rats were divided randomly into two groups. In one group of four rats, phentolamine (30 μg in 10 μl of saline followed by a flush with 10 μl of saline) was delivered intrathecally immediately after the termination of EA, which was applied to the SI-6 point for 30 min. In the other group of three rats, saline (20 μl) was injected intrathecally immediately after the termination of EA application. WBF was measured at 0.5, 1, 2, and 4 h after drug treatment. The next day, the procedures for these two groups were reversed. Therefore, all seven rats received phentolamine and saline injections over a 2-day period in random order. The pre-EA baseline values were measured before each experiment, and data were expressed as the percentage of maximum recovery as shown in Fig. 5A. The data were analyzed using a cross-over repeated ANOVA model and fixed factors, 'injection sequence', 'post-injection time', and 'treatment group', were fitted to the data with the correlated error term. The analysis showed that the treatment group factor for the two groups was significantly different ($F = 20.67$, $p = 0.0003$), whereas the factor for the injection sequence was not ($F = 0.08$, $p = 0.782$). These data indicate that intrathecal administration of phentolamine completely blocked the EA-induced analgesic effect, suggesting that EA-induced analgesia in ankle sprain pain is mediated by spinal α-adrenoceptors.

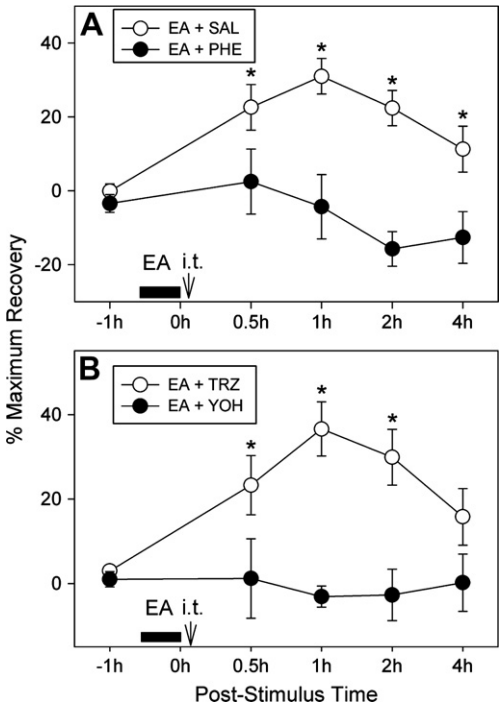

Fig. 5. Effects of intrathecal application of α-adrenergic receptor antagonists on EA-induced analgesia in rats with sprained ankles. Graphs are plotted for the average values (±SEM) of percentage of maximum recovery. (A) Effects of intrathecal application of a mixed α-adrenergic receptor antagonist on EA-induced analgesia ($n = 7$ rats). Phentolamine (30 μg in 10 μl of saline) or the same volume of saline was given intrathecally (through an implanted catheter) immediately after termination of EA (applied to the SI-6 point on the contralateral forelimb for 30 min). The experiment was done with the cross-over design so that all seven rats received both phentolamine and saline alone in a 2-day span (1st and 2nd post-sprain days). There was no significant improvement in WBF in the phentolamine-injected group, whereas the saline injection group showed a long-lasting recovery of WBF. An asterisk (*) indicates a value significantly ($p < 0.05$) different from the equivalent value after phentolamine injection, using cross-over repeated ANOVA with the least square method. (B) Effects of intrathecal application of specific α_1- and α_2-adrenergic receptor antagonists on EA-induced analgesia ($n = 8$ rats). Yohimbine (an α_2-adrenoceptor blocker, 10 μg/10 μl of saline) or terazosin (an α_1-adrenoceptor blocker, 10 μg/10 μl of saline) was given intrathecally through an implanted catheter immediately after termination of EA (applied to the SI-6 point on the contralateral forelimb for 30 min). The experiment was done with the cross-over design so that all eight rats received both yohimbine and terazosin in a 2-day span (1st and 2nd post-sprain days). There was no significant improvement in WBF in the yohimbine-injected group, whereas the terazosin injection group showed a long-lasting recovery of WBF. An asterisk (*) indicates a value significantly ($p < 0.05$) different from the equivalent value after yohimbine injection, using cross-over repeated ANOVA with the least square method.

3.5. Effect of intrathecal injection of α-adrenergic antagonist subtypes on EA-induced analgesia

Because our data indicated that spinal α-adrenoceptors may be involved in mediating EA-induced analgesia, we investigated the subtype of α-adrenoceptors that may be involved by using specific α_1- and α_2-adrenoceptor antagonists. Intrathecal catheters were implanted in eight rats. One week later, the ankles of these rats were sprained. Experiments were again conducted in cross-over design with injections of compounds in the 2-day period after ankle sprain. One day

after ankle sprain, eight rats were divided randomly into two groups of four. In one group, terazosin (an α_1-adrenoceptor antagonist; 10 μg/10 μl saline) was injected intrathecally immediately after the termination of EA application at the SI-6 point. In the other group of rats, yohimbine (an α_2-adrenoceptor antagonist; 10 μg/10 μl of saline) was injected intrathecally immediately after the termination of EA. The WBF of the affected hind limb was measured before and at 0.5, 1, 2, and 4 h after the drug treatment. The next day, the procedures for these two groups were reversed. Therefore, all eight rats received both terazosin and yohimbine over a 2-day period in random order. The data were expressed as the percentage of maximum recovery as shown in Fig. 5B. The data were analyzed using a cross-over repeated ANOVA model and fixed factors, 'injection sequence', 'post-injection time', and 'treatment group', were fitted to the data with the correlated error term. The analysis showed that the treatment group factor for the two groups was significantly different ($F = 23.31$, $p < 0.0001$), whereas the factor for the injection sequence was not ($F = 1.44$, $p = 0.243$). These data indicate that the EA-induced analgesic effect was completely blocked by intrathecal yohimbine treatment, but not by terazosin injection, suggesting that EA-induced analgesia in ankle sprain pain is mediated by spinal α_2-adrenoceptors.

The effect of lower doses of intrathecal yohimbine on EA-induced analgesia in ankle sprain pain was also examined in another group of seven rats. Intrathecal injection of 1 μg of yohimbine produced a negligible effect ($n = 6$), while 5 μg of yohimbine ($n = 7$) slightly reduced EA-induced analgesia, although the reduction was not statistically significant.

We also tested intrathecal injection of idazoxan, another type of α_2-adrenoceptor antagonist, on EA-induced analgesia in ankle sprain pain. In four rats, injection of 30 μg (in 10 μl of saline) almost completely blocked EA-induced analgesia (percentage maximum recovery values at 1 and 2 h after injection were 6.9 ± 5.0 and -1.6 ± 8.1, respectively).

4. Discussion

The present study examined the neurotransmitters involved in electroacupuncture-induced analgesia in ankle sprain pain in rats. After ankle sprain, rats decrease weight bearing on the affected hind limb. EA treatment produces temporary reversal of this effect, suggesting that EA has an analgesic effect. This EA-induced analgesia was completely blocked by a systemic injection of the α-adrenergic antagonist phentolamine or by an intrathecal injection of the α_2-adrenoceptor antagonist yohimbine, but not by the α_1-adrenoceptor antagonist terazosin. The data suggest that EA-induced analgesia is mediated by α_2-adrenoceptors in the spinal cord.

The involvement of the endogenous opioid system is a well-established hypothesis for the explanation of acupuncture analgesia. Several previous studies, however, have indicated that opioid antagonists have failed to interfere with acupuncture analgesia under certain circumstances (McLennan et al., 1977; Chapman et al., 1980, 1983; Takeshige et al., 1980, 1992; Bossut et al., 1991; Koo et al., 2002). In fact, a recent study (Harbach et al., 2006) concluded that β-endorphin is released by stress but not by the acupuncture procedure per se during acupuncture treatment. On the other hand, there is evidence suggesting that non-opioid systems may be involved in EA-induced analgesia. For instance, administration of 5-HT or catecholamine antagonists or depletion of cellular monoamine content blocked the EA-induced analgesic effect, but an opioid antagonist failed to do so (Takeshige et al., 1980; Cheng and Pomeranz, 1981). The antinociceptive effect produced by chemical stimulation of an acupuncture point was also blocked by an α_2-adrenoceptor antagonist but not by an opioid antagonist in a visceral pain model (Kwon et al., 2001). These results suggest the possible involvement of the monoaminergic system, noradrenergic and/or serotonergic, in the mediation of acupuncture analgesia. It appears that the analgesic effect induced by acupuncture in various conditions may be mediated by different mechanisms depending on the specific conditions. It is also possible that stimulation of different acupuncture points may trigger different mechanisms. Future systematic studies are warranted to resolve these issues.

The present study tested the possible involvement of the monoaminergic system in EA-induced analgesia in ankle sprain pain because an opioid antagonist, naloxone, was not effective in blocking EA-induced analgesia. This study confirms our previous results of failed attempts to block EA effect by opioid antagonists, both naloxone and naltrexone (a longer-lasting opioid antagonist) (Koo et al., 2002). The descending adrenergic pathway is considered one of the major spinal analgesic systems originating from the brainstem (Yaksh, 1985; Proudfit, 1988; Millan, 2002). Thus, release of spinal norepinephrine (NE) and activation of spinal α_2-adrenoceptors represent important components of descending control of nociception. Many studies have shown a role of the adrenergic descending pathway in the modulation of nociception. For example, spinal administration of NE (Reddy et al., 1980; Yaksh, 1985; Eisenach et al., 1996; Shinomura et al., 1999) or electrical stimulation of central noradrenergic cells induced powerful antinociception (Stamford, 1995; Nuseir and Proudfit, 2000). Further studies found that the antinociceptive effect of NE is mediated by α_2-adrenoceptors (Howe et al., 1983; Fleetwood-Walker et al., 1985; Yaksh, 1985). The activation of α_2-adrenoceptors increases potassium

conductance in dorsal horn neurons, which produces hyperpolarization and decreases excitability, thereby contributing to analgesia (North and Yoshimura, 1984; Ocana and Baeyens, 1993; Grudt et al., 1995). On the other hand, α_2-adrenoceptor agonists reduce the stimulus-evoked release of substance P (Kuraishi et al., 1985; Pang and Vasko, 1986) and glutamate (Kamisaki et al., 1993; Ueda et al., 1995) in the spinal cord, thus suppressing nociceptive transmission. The locus ceruleus in the brainstem is a major cell group of catecholaminergic neurons and is known to project to the spinal cord. Antinociception induced by stimulation of the locus ceruleus is reduced by intrathecal injection of a non-selective adrenergic antagonist, phentolamine, or a selective α_2 antagonist, yohimbine, but not by a selective α_1 antagonist, prazosin (Jones and Gebhart, 1986). The fact that the analgesic effect induced by EA was reversed by phentolamine or yohimbine, but not by terazosin, clearly agrees with previous studies, finding that analgesia is mediated by α_2-adrenoceptors. In addition, there have been reports that stimulation of peripheral Aδ and C fibers activates the descending adrenergic system and releases NE in the spinal cord (Tyce and Yaksh, 1981; Men and Matsui, 1994). Thus, we propose that EA activates noradrenergic bulbospinal neurons, resulting in spinal NE release and activation of dorsal horn α_2-adrenoceptors. Thus, NE suppresses noxious inputs from the ankle, restoring weight bearing in the affected foot of rats in this model.

The present study used various pharmacological receptor antagonists. Specificity and appropriate dose are important issues in such studies, particularly for antagonists with negative results. We used 1 mg/kg of naloxone, which is a comparable systemic dose reported to block acupuncture analgesia in rodent in a previous study (Pomeranz and Chiu, 1976). In our previous study (Koo et al., 2002), we also tested a much higher dose (10 mg/kg) of naltrexone, which was confirmed to block the effect of 5 mg/kg of morphine. Therefore, we feel that the negative result using the opioid receptor antagonist is valid. There are fewer problems with phentolamine or yohimbine because they were effective at the doses used. Although we primarily used yohimbine as an α_2-adrenoceptor blocker, intrathecal injection of idazoxan, another type of α_2-adrenoceptor selective antagonist, also blocked EA-induced analgesia. We used terazosin as a selective α_1-adrenoceptor antagonist rather than the perhaps more commonly used prazosin to test the reversal of the EA effect in ankle sprain pain. The reason is that terazosin produces a longer-lasting effect (Howe et al., 1983; Akduman and Crawford, 2001), and our previous studies suggest that an effective dose of terazosin is equivalent to that of the α_2-adrenoceptor blockers (yohimbine and idazoxan) (Lee et al., 1999; Moon et al., 1999). These results strongly suggest that EA-induced analgesia is mediated by α_2-adrenoceptors in the spinal cord.

In summary, we have demonstrated a powerful analgesic effect of EA applied to the contralateral forelimb in rats with ankle sprain. This analgesic effect was reversed by spinal application of α_2-adrenoceptor antagonists, but not by a systemic opioid antagonist. These data suggest that EA-induced analgesia in ankle sprain pain is mediated by spinal α_2-adrenoceptors. Further study is needed to identify which brain region is activated by EA stimulation in ankle-sprained rats to release norepinephrine in the spinal cord.

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