Electroacupuncture reduces the evoked responses of the spinal dorsal horn neurons in ankle sprained rats

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ABSTRACT

Acupuncture is shown to be effective in producing analgesia in ankle sprain pain in humans and animals. To examine the underlying mechanisms of the acupuncture-induced analgesia, the effects of electroacupuncture (EA) on weight bearing forces (WBR) of the affected foot and dorsal horn neuron activities were examined in a rat model of ankle sprain. Ankle sprain was induced manually by over-extending ligaments of the left ankle in the rat. The dorsal horn neuron responses to ankle movements or compression were recorded from the lumbar spinal cord using an in-vivo extracellular single unit recording set-up, one day after ankle sprain. EA was applied to the SI-6 acupoint on the right forelimb (contralateral to the sprained ankle) by trains of electrical pulses (10 Hz, 1 ms pulse width, 2 mA intensity) for 30 minutes. After EA, WBR of the sprained foot was recovered significantly and dorsal horn neuron activities were suppressed significantly in ankle sprained rats. However, EA produced no effect in normal rats. The inhibitory effect of EA on hyperactivities of dorsal horn neurons of ankle sprained rats was blocked by the alpha adrenoceptor antagonist, phentolamine (5 mg/kg, i.p.) but not by the opioid receptor antagonist, naltrexone (10 mg/kg, i.p.). The data suggest that EA-induced analgesia in ankle sprain pain is mediated mainly by suppressing dorsal horn neuron activities through alpha-adrenergic descending inhibitory systems at the spinal level.

Keywords: acute ankle sprain, electroacupuncture, α-adrenergic descending inhibition
Clinical and experimental studies have suggested that acupuncture at certain acupoints has therapeutic effects in various painful conditions (Kim et al. 2010; Manheimer et al. 2010). It has been reported that the number of individuals who received alternative medical care was estimated to be 2.1 million in America in 2002 (Tindle et al. 2005). The use of acupuncture treatment for joint sprains, in particular, increased approximately 30% between 1990 and 1997 (Eisenberg et al. 1998). Thus there is an increasing trend of using acupuncture for pain caused by musculoskeletal problems. According to the survey of American physicians who utilize alternative medical treatments, 76% of them have used acupuncture treatment for patients with ankle sprain and 88% of them indicated that acupuncture was effective (Diehl et al. 1997). While acupuncture is gaining popularity as a therapeutic intervention in the United States, the efficacy or therapeutic benefits are still debatable (Linde et al. 2005; Manheimer et al. 2009; Vickers et al. 2004a, 2004b) and the underlying mechanisms are not well understood.

Ankle sprain is one of the most common sports-related injuries (roughly 25% of all sports-related injuries) and involves trauma to the lateral ankle ligament complex (Hume and Gerrard 1998; Puffer 2001). Approximately one third of the subjects that once had ankle sprain, later experience recurring symptoms, such as swelling and persistent pain (Konradsen et al. 2002). Two separate components can contribute to persistent pain after ankle sprain: sensitization of nociceptors due to joint inflammation (peripheral sensitization) and sensitization of dorsal horn neurons (central sensitization) following intense nociceptive inputs. The contribution of peripheral sensitization in joint inflammation has been studied extensively and both inflammatory agents from damaged tissues and/or activated fine sensory fibers (neurogenic
inflammation) (Ferrell et al. 1997; McDougall et al. 1997, 2006; Schaible et al. 2009) have been well documented. On the other hand, the central mechanism of ankle sprain pain has received much less attention.

In a previous study, our laboratory developed a rat model of ankle sprain with the severity equivalent to Grade I or II of human ankle sprain (Koo et al. 2002). In this model, electroacupuncture (EA) applied to the SI-6 acupoint on the contralateral forelimb produced a significant analgesic effect (Koo et al. 2002). The behavioral studies with pharmacological manipulations indicated the analgesic effects of EA on ankle sprain pain were through the noradrenergic system (Kim et al. 2010; Koo et al. 2002, 2008). The aim of this study was to determine the effect of EA on the dorsal horn neuron responses to ankle movements, using the characteristics of these neurons in normal and acute ankle sprained rats described in the companion paper (Kim et al. 2011). In addition, the parameters of effective EA and specific acupoints that produce changes in dorsal horn neurons excitability were examined.

MATERIALS AND METHODS

Experimental animals

Adult male Sprague-Dawley rats (Harlan Sprague-Dawley Co., Houston, TX) were used for this study (200–350g body weight). The rats were divided into two groups: normal and acute ankle sprained. All experiments were carried out in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals and the animal protocol approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch.
Animals were housed in plastic cages with soft bedding and were provided with free access to food and water under a 12/12 h reversed light-dark cycle. All animals were adapted for 7 days before the experiment.

**Induction of ankle sprain**

Ankle sprain was induced manually as described previously (Koo et al. 2002; Kim et al. 2010) under halothane anesthesia (3% in air for induction and 1.5 ~ 2% for maintenance). Briefly, the left hind foot was overextended repeatedly in the direction of simultaneous inversion and plantar flexion for 60 times during a 1 min period with gradual increase of bending force. The same procedures were repeated one more time. At the end, the ankle could be rotated to a position of 180° inversion. Anesthesia was discontinued and the rats recovered within 5-10 min. The resulting ankle sprain was similar to a Grade I to II in humans with stretched and/or partially torn ligaments without complete rupture (Cotler 1984; Koo et al. 2002).

**Behavioral testing**

To estimate the level of pain in ankle sprained rats, the amount of weight bearing force on the affected foot was measured on 1 day after the ankle sprain. On the day of behavioral testing, each rat was weighed and then allowed to walk through a long rectangular plastic tube (10 cm width, 10 cm height, and 60 cm length) with a scale (Acculab Pocket Pro 250-B, Newton, PA, USA) located midway down the tube that only underlay the right-hand half of the tube. Thus, when the rat walked in one direction the weight on the left foot could be determine and when the rat came down the other way, the weight placed on the right foot could be measured. The weight signals from the scale were fed into an oscilloscope and the weight bearing forces were
calculated using a data acquisition system (CED 1401 plus with Spike 2 program, CED, UK). To obtain a valid weight bearing force of the foot, weight bearing forces were measured 6 times and the values were averaged for each measurement.

Drug administration

Intraperitoneal injections of two pharmacological receptor blockers were tested on EA induced effects on weight bearing forces and dorsal horn activities. These were an opioid receptor blocker, naltrexone hydrochloride (10 mg/kg, Sigma, St. Louis, MO, USA) and an \( \alpha \)-adrenoceptor antagonist, phentolamine hydrochloride (5 mg/kg, Sigma, St. Louis, MO, USA).

Electroacupuncture (EA)

The results of our previous studies showed that the contralateral SI-6 is the acupoint where the most powerful EA-induced analgesic effect was produced in ankle sprain pain (Koo et al 2002; 2008). Thus we chose this acupoint to study the EA effect on pain behaviors as well as responses of dorsal horn neurons to ankle manipulations. In addition, the ST-36 acupoint that did not produce analgesic effect on ankle sprain pain was used as a control EA point.

Under general anesthesia (halothane 1.0 - 1.5%), a pair of stainless steel needles (0.3 mm in diameter) separated by 1 mm were inserted transcutaneously to a depth of 5 mm into either the SI-6 acupoint (Yanglao, the dorso-lateral aspect of the head of ulna) of the contralateral forelimb or the ST-36 acupoint (Zusanli, on the proximal part of the tibialis anterior muscle ~10 mm below the knee joint) of the ipsilateral hind limb. For EA, various trains of pulses (2, 10 or 100 Hz, 1 ms pulse width, 2 mA intensity [ten times of the muscle twitch threshold]) were applied to the inserted needles for 30 minutes with an electrical stimulator (A300 & A385, WPI, USA). The
delivered current was monitored at all times and the polarity was reversed every 60 sec to prevent polarization of the electrodes. Immediately after the termination of EA, anesthesia was discontinued and the rats usually resumed full activity within 5-10 min. For the control rats, needles were inserted at the same acupoint but electrical stimulation was not applied. Behavior tests were done 30, 60, 120 and 240 min after termination of EA.

Surgical preparations for electrophysiological recording

Recordings were performed in normal (n=17) and ankle sprained rats one day after ankle sprain (n=30). Rats were anaesthetized with urethane (1.5 g/kg, i.p.) and supplemental urethane (200 mg/kg, i.p.) was given when a sign of low anesthesia level was detected. The depth of anesthesia was monitored by observing the heart rate, the end-tidal CO₂ level, and the pupil size. The trachea was cannulated to ensure adequate respiration. The rat was paralyzed with pancuronium bromide (i.v., 1 mg/kg for initiation and 0.4-0.6 mg/kg/h for maintenance) and artificially ventilated at a rate of 80-100 breaths/min to maintain the end tidal PCO₂ levels at 3.5-4.5%. A laminectomy was done to expose L4-L6 spinal cord segments and then the rat was secured in a stereotaxic frame. The vertebral column was held tight by clamps caudal and rostral to the laminectomy region and the dura was opened. The spinal cord was continually bathed in a pool of warm mineral oil. The core body temperature was monitored and maintained at 36.5-37 °C by a heating blanket connected to a rectal thermal probe via an automatic feedback control unit. At the end of the experiment, rats were euthanized with an overdose of urethane (3 g/kg) and death was confirmed by opening the chest.

Recording of spinal dorsal horn neurons
In ankle sprained rats, extracellular single cell recordings were made from dorsal horn neurons in the spinal cord ipsilateral to the sprained ankle. Control recordings were made from those in normal rats. The recordings were made with a carbon fiber micro-electrode (0.4-0.8 MΩ; Kation Scientific, MN, USA). The electrode was lowered into the cord using an electronic micromanipulator (Burigh), which allowed us to measure the depth of the recording site from the dorsal surface. Electrophysiological activities of dorsal horn neurons were fed into a pre-amplifier and an amplifier (CYBERAMP 320, Axon Ins., Foster City, CA), and were displayed on an oscilloscope. The amplified signals were also fed into a window discriminator (WPI, USA) and a Spike-2 program (version 4, CED, UK) to isolate single unit recordings. Responses to ankle movement stimuli were stored in a computerized data acquisition system (CED140l, CED, UK) for further analysis. To examine EA induced neuronal changes, EA was applied to the contralateral SI-6 acupoint at 10 Hz, 1 ms pulse width and 2 mA for 30 minutes.

**Mechanical stimulations to the ankle**

Three different types of stimuli were used to activate deep sensory fibers in the ankle joint: plantarflexion, inversion, and compression. A special device equipped with a linear potentiometer was patched to the plantar surface of the rat hind paw and used to measure movement angles. For plantarflexion stimulation, the foot was first brought to the tibiotarsal-tarsometatarsal (TT-TM) angle of 90° as the starting point and then moved the foot to the 190° angle, the maximum plantarflexed position. For inversion stimulus movement, the foot was rotated medially from the resting position (0° on horizontal plane) up to 120°. For each movement stimulus, the foot was slowly moved from the starting position to the maximally
moved position during the first 2 seconds, held at the maximally moved position for 12 seconds, and then moved back to the starting position during the last one second. The range of each movement was set where the joint showed appreciable resistance felt at the maximum point but did not induce excessive tissue injury. For compression stimulus, the pressure was applied medio-laterally to the ankle by using a pair of large blunt forceps (20 cm long; contact area, 4 mm x 4 mm) equipped with strain gauges (Yu et al. 2002). Each compression stimulus started from 0 g intensity but quickly reached to 1500 g within 2 sec and then held at the maximum intensity for 13 sec and then quickly released.

**Experimental design for evoked neuronal response measurement**

The deep dorsal horn neurons responding exclusively to plantarflexion, inversion, or compression stimuli to the ankle were examined. The neurons responding to cutaneous brush and/or pinch stimuli of the foot skin were thus excluded from the study. Once the neuron which responds to a specific stimulus was identified, the spontaneous activities were recorded for 60 sec prior to foot stimulation. These ‘background activities’ were then subtracted from the responses to each stimulus. To establish reliable responses of each neuron, neuronal responses were recorded initially 2-3 times by repeating the same mechanical stimulus at 10-min intervals. When the responses to each stimulus produced no more than 10% variations from the original, they were considered as stable responses. Once the stable responses were recognized, the recordings were made 3 times with the same stimulus repeated 3 times and the average of the 3 recordings was used as the average response to that stimulus.

**Data analysis and statistics**
Three different response parameters were analyzed: peak evoked responses, mean evoked responses, and after-discharges. All evoked responses were adjusted by subtracting the averaged background discharges that were recorded without mechanical stimuli. The peak evoked response values were the maximum response values during the entire period of each stimulus. The mean evoked response values were the averaged evoked discharges during the entire period of mechanical stimulation. Some neurons maintained increased activities after the stimuli were removed and those were considered as after-discharges. When after-discharges were present, those activities were recorded for 20 sec after removal of the stimulus and then the averaged values were presented in the data. The peak and mean neuronal response were measured 3 times at 10 min intervals and then averaged values of those 3 were normalized as 100% and used as the baseline values before EA application. All data are presented as the mean ± S.E. Statistical significance was analyzed using Two-way ANOVA with one repeated factor followed by the Duncan’s multiple comparison post-hoc test, using the program Sigmastat (ver. 3.0, SPSS, USA). P values less than 0.05 were considered to be significant.

RESULTS

1. EA at SI-6 produced analgesic effects through spinal adrenergic systems

Three separate features of EA induced analgesia were tested in this series of experiments: point specificity, stimulus parameters, and potential neurotransmitters involved. To test the point specificity, the effect of EA applied to 2 different locations, the SI-6 or ST-36, on weight bearing forces on the ankle sprained hind limb was examined and the data are shown in Fig. 1A. Since
our previous studies showed that the contralateral SI-6 acupoint produced powerful EA-induced analgesic effect in ankle sprain pain (Koo et al. 2002, 2008), we chose this acupoint to study the EA effect on pain behaviors as well as responses of dorsal horn neurons to ankle manipulations. The data are presented as percent changes of weight bearing forces before and after EA application. The weight bearing forces one day after acute ankle sprain and immediately before EA application were used as a baseline value. Thirty minutes after EA application (1 msec. pulses, 2 mA, 10 Hz, for 30 min) at the contralateral SI-6 acupoint, the weight bearing forces of the affected limb increased significantly and this analgesic effect lasted at least 4 hours. On the other hand, the EA applied to the ipsilateral ST-36 acupoint did not show significant change. Thus, the data indicate that there is a specificity of acupoint which induces analgesic effect on ankle sprain pain.

To test whether EA analgesic effect depends on stimulus parameters, the effects of EA with 3 different frequencies were tested. EA was applied to the contralateral SI-6 acupoint and then weight bearing forces were measured before and several times after EA application. The data of this experiment are shown in Figure 1B. EA with 10 Hz stimulus frequency was the most effective in increasing the weight bearing forces on the affected foot as compared to that with 2 Hz or 100 Hz. The data indicate that the stimulus parameter of EA is critical for generation of effective analgesia and 10 Hz seems to be the optimum frequency of stimulation.

The third experiment was to identify the possible endogenous inhibitory system involved in EA analgesia. The possible involvement of two endogenous inhibitory systems (opioid and adrenergic) was examined using pharmacological receptor blockers. Either naltrexone (10 mg/kg), an opioid receptor antagonist, or phentolamine (5 mg/kg), an α-adrenoceptor antagonist, was administered intraperitonially 30 min before EA application. EA (1 msec. pulses, 2 mA, 10
Hz) was applied to the contralateral SI-6 point for 30 min and weight bearing forces were measured before and several times after the EA application. As shown in Fig. 1C, a systemic naltrexone did not block the EA induced increase in the weight bearing forces of the ankle sprained foot. On the other hand, a systemic phentolamine significantly reduced the EA induced increase in the weight bearing forces. The data suggest that EA induced analgesia is mediated by the endogenous adrenergic inhibitory system and not by the opioid inhibitory system.

2. Dorsal horn neuron responses were reduced after EA

Extracellular recordings were made from the deep dorsal horn (lamina IV-VII) neurons that respond to a specific ankle stimulus. The recordings were made 30 min before, during, and 0, 15, 30, and 60 min after the termination of EA application (shaded region in graphs) at the SI-6 acupoint for 30 min (Figs. 2-4). The responses were recorded in a total of 36 neurons (1 neuron per rat) from 17 normal and 19 ankle sprained rats (one day after ankle sprain). As in the study of the companion paper (Kim et al. 2011), the majority of neurons in both the normal and sprained rats responded exclusively to a specific afferent modality: plantarflexion, inversion, or compression. The neurons responding to multiple stimuli were rare and were excluded in this study.

The EA effects on the activities of plantarflexion (PF) neurons from 6 normal and 6 ankle sprained rats are shown in Fig. 2. The examples of actual recordings are shown in Figs. 2A & 2B and the summary of response changes is shown in Figs. 2C & 2D. In normal rats, PF neurons showed strong evoked activity in response to plantarflexion and the activity stopped when the foot was returned to resting position (Fig. 2A). Furthermore, the response activities to PF
movement did not change either during or after EA application in normal rats (Figs. 2A, 2C, and 2D). On the other hand, in ankle sprained rats, EA application significantly reduced the response activities of PF neurons along with after-discharges (Figs. 2B, 2C and 2D). The EA-induced inhibition lasted for at least 30 min after the termination of EA.

The activities of the dorsal horn neurons responding to inversion of the foot were recorded in 5 neurons in normal rats and 5 neurons from rats one day after ankle sprain. The typical response patterns are shown in Fig. 3A for normal and in Fig. 3B for ankle sprained rats. The averaged data are also shown in Figs. 3C and 3D. Similar to plantarflexion neurons, EA produced inhibition of evoked response of inversion neurons recorded from ankle sprained rats but not from those recorded from normal rats. The after-discharges (which developed in ankle sprained rats) were also significantly reduced after EA for a long period of time.

The activities of the dorsal horn neurons responding to ankle compression were recorded in 6 neurons in normal rats and 8 neurons in ankle sprained rats. The typical response patterns are shown in Fig. 4A for normal and in Fig. 4B for ankle sprained rats. The averaged data are also shown in Figs. 4C and 4D. Again, the response pattern to ankle compression was similar with the study done in the companion paper (Kim et al. 2011). As with plantarflexion and inversion neurons, EA produced inhibition of the evoked responses of compression neurons as well as after-discharges.

3. Phentolamine blocked EA induced reduction of dorsal horn neuron responses

The behavioral study showed that the EA induced analgesia was partially blocked by phentolamine (Fig. 1C), thus suggesting EA induced analgesia may be mediated by α-adrenoceptors. To confirm this in neuronal activity, the effect of phentolamine on dorsal horn
neuron responses was examined. In this part of the study, activities of the dorsal horn neurons that respond to plantarflexion (n=4), inversion (n=3) or compression (n=4) were recorded in ankle sprained rats before, during and after EA application with or without phentolamine administration (5 mg/kg, i.p.). Two examples of recordings are shown in Fig. 5A (without phentolamine) and Fig. 5B (with phentolamine injection 60 min before EA application). Because EA-induced inhibitions were similar among the neurons responding to plantarflexion, inversion or compression, the results were pooled together from all 11 neurons recorded regardless of stimulus modality and the summary data are shown in Figs. 5C and 5D. As shown in Fig. 5A, EA produced a significant inhibition of the dorsal horn neuron responses to inversion without phentolamine treatment. On the other hand, when the rat was pretreated with phentolamine, EA no longer produced the inhibition (Fig. 5B). The summary data also show that the mean responses and after-discharges were significantly reduced by EA (4 neurons, open circle) but this EA inhibition was completely blocked by phentolamine pretreatment (7 neurons, filled circle). In contrast, the dorsal horn neuron responses to ankle stimuli recorded in the normal rat were not affected either by EA application or with the same dose of phentolamine. Thus, the data suggest that EA-induced inhibition of dorsal horn neuron responses to ankle stimuli is mediated by α-adrenoceptors, confirming the result of the behavioral study.

DISCUSSION

The present study examines EA effect on the activities of dorsal horn neurons in ankle sprained rats and compares this to the EA effect on pain behaviors. The activities of the deep dorsal horn neurons responding to plantarflexion, inversion or compression were significantly
reduced by EA application to the contralateral SI-6 acupoint in ankle sprained rats. The EA stimulus parameter of 10 Hz was more effective to induce analgesia than either a lower (2 Hz) or a higher (100 Hz) frequency. In addition, α-adrenoceptor antagonist phentolamine significantly reduced both EA-induced analgesia and dorsal horn neuron responses. Thus, the data suggest that the reduction of dorsal horn neuron activities is the neural substrate for EA-induced analgesia.

In general, ankle sprain involves a stretch or tear of the lateral ligament complex of the ankle joint, the anterior and posterior talofibular and the calcaneofibular ligaments, during an unanticipated sudden hyper-plantarflexion and inversion (Safran et al. 1999). Although traditional conservative therapies on ankle sprain are quite effective (Karlsson 1998), about a third of patients suffer from persistent pain, swelling or recurrent sprain (Anandacoomarasamy and Barnsley 2005; Kern-Steiner et al. 1999; Konradsen et al. 2002; Koo et al. 2002). Recent surveys indicate that acupuncture has been used for treating musculoskeletal disorders (Cai 2010; Freedman 2002; Song 1993; Yang et al. 2009; Yao-chi et al. 2007; Zhou and Liu 2003), including ankle sprain (Diehl et al. 1997; He and Xu 2006; Park et al. 2004; Zhang and Miao 1990). Although the popularity of acupuncture has increased in recent years, its underlying mechanism is not clear. The main purpose of this study is to establish the basis of neural mechanisms of the effect of acupuncture in ankle sprain pain. Following the study in the companion paper (Kim et al. 2011), which characterized the response properties of the dorsal horn neurons to ankle stimulation, the present study examines their responses to EA application. The present study shows that EA produced a significant reversal of enhanced evoked responses as well as after-discharges developed in ankle sprained rats. Thus the data indicate that ankle
sprains sensitize dorsal horn neurons and this sensitization is reversed by EA. On the other hand, EA does not produce any effect on normal dorsal horn neuron responses.

Although it is clear that EA transiently reverses sensitization of dorsal horn neurons, the mechanism underlying how EA produces such a reversal is not clear. The fact that EA applied to the forelimb produces its action on the lumbar dorsal horn neurons suggests that one or more endogenous descending inhibitory systems are involved. One well known such system is the opioid system, thus EA may have produced its action through the endogenous opioid inhibitory system. In fact, several previous studies indicated that EA induced analgesia might have been mediated by the opioid system (Han 2003; Kim et al. 2004; Mayer et al. 1977; Wang et al. 2008; Zhang et al. 2004a) since EA analgesia was blocked by opioid receptor antagonists. On the contrary, several other studies suggested that EA-induced analgesia is mediated by the adrenergic inhibitory system or non-opioid system (Kim et al. 2005; Kim et al. 2010; Koo et al. 2002, 2008; Laitinen 1982; Zhang et al. 2004b). If the opioid system is the main mechanism underlying EA-induced analgesia in ankle sprained pain, we would have expected that the opioid receptor antagonist, naltrexone, would have a significant reduction in EA-induced analgesia. However, the behavioral data showed that naltrexone had a minimum effect. On the other hand, an α-adrenoceptor antagonist, phentolamine, produced a significant reduction in EA-induced analgesia, suggesting that adrenoceptors are importantly involved in EA-induced analgesia. Similarly, enhanced responses of the dorsal horn neurons after ankle sprain were significantly reduced by EA. This EA-induced reduction in the dorsal horn neuron responses was also blocked by phentolamine. Thus, both the behavioral studies and the electrophysiological studies suggest that the endogenous descending adrenergic system could be involved in EA-induced analgesia in ankle sprain pain and the system is mediated by α-adrenoceptors. Another possibility is that the
phentolamine effect is through either peripheral or presynaptic α-adrenoceptors (Moon et al. 1999). Considering that EA-induced analgesia in ankle sprain pain is almost completely inhibited by intrathecal phentolamine (Koo et al. 2008), it is likely that this phentolamine effect is primarily through spinal actions. Furthermore, the presence of presynaptic α-adrenoceptors in the spinal cord has not been shown. Thus, we speculate that EA induced analgesia in ankle sprain pain is mediated by the descending adrenergic system and postsynaptic α-adrenoceptors.

The reasons why some EA-induced analgesia is blocked by α-adrenergic antagonists while others are more affected by opioid inhibitors are not clear. The possible factors include: 1) EA applied to different acupoints may activate different inhibitory systems; 2) EA may activate multiple inhibitory systems and different pain conditions (and animal models) may require different inhibitory systems to produce analgesia; and/or 3) different EA stimulus parameters may activate different inhibitory systems. Further studies are warranted to sort out these and other possibilities of the endogenous inhibitory systems that are involved in EA-induced analgesia.

Our data showed that stimulus frequency of 10 Hz is most effective in producing analgesia in ankle sprain pain compared to 2 Hz or 100 Hz. Thus, the present results suggest that the efficacy of EA-induced analgesic effect is dependent on stimulus parameters. In previous studies, different analgesic effects were induced depending on the stimulus parameters with EA (Hahm 2007; Lao et al. 2004; Park et al. 2006; Yang et al. 2010) or transcutaneous electrical nerve stimulation (TENS) (Sluka et al. 2000) in animals. The reasons for the differential efficacy of different stimulus parameters are not entirely clear. One possibility is that different stimulation frequencies may activate different sets of primary afferents and thus transmit inputs to different parts of the central nervous system. Another possibility is that a certain frequency of stimulus is
more effective in activating a specific population of afferent fibers, which initiate analgesic
effect. Further study is warranted to identify the most effective stimulus parameters of EA and
the underlying mechanisms of the differential efficacy.

Traditionally, acupuncture treatment involves insertion of needles to a certain depth at
specific acupoints designated to specific diseases, suggesting the existence of point-specificity of
acupoints (Zaslawski et al. 2003). The present study shows that EA at contralateral SI-6, but not
ipsilateral ST-36, is effective in producing analgesia in ankle sprained rats. It is not known the
exact nature of acupoints. A handful of studies, however, suggested that the distribution of
acupoints is related to peripheral nerve routes and, thus, performing acupuncture is a form of
stimulation of a specific peripheral nerve. For example, low frequency EA applied to the ST-36
acupoint produced μ-opioid dependent analgesia (Han 2003) and the ST-36 is located on the
tibial nerve route. Furthermore, a low frequency stimulation of the tibial nerve has shown to
inhibit the activities of spinothalamic tract neurons in an opioid dependent manner in primates
(Chung et al. 1984a, 1984b). Thus it is possible that the specificity of EA is related to the
stimulation of a specific peripheral nerve located at that acupoint. While we still use specific
acupoints to treat certain diseases based on previous records or experience, the identity of
acupoints and their mechanism of producing specific outcomes need to be explored in the future.

It is interesting to note the difference between opioid and α-adrenoceptor mediated
analgesic systems. Both morphine (the accompanying study) and EA (the present study) reduce
responses of dorsal horn neurons to ankle stimulation. However, the two are mediated by
different mechanisms since naltrexone reverses morphine but not EA-induced reduction of dorsal
horn neuronal responses. Therefore, analgesic effects can be achieved by multiple mechanisms,
such as opioid receptor and α-adrenoceptor mediated. However, EA-induced analgesic effect in
ankle sprain pain seems to be mediated by one of such mechanisms, the $\alpha$-adrenoceptor mediated analgesic system.

In conclusion, the study demonstrated that EA at the SI-6 acupoint in the contralateral forelimb produces a long lasting inhibition of responses of dorsal horn neurons to ankle stimulation in ankle sprained rats. The suppression was likely accomplished through activation of a descending adrenergic inhibitory system and $\alpha$-adrenergic receptors in the spinal cord. Future studies are warranted for delineation of much detail mechanisms including exploration of supraspinal origin of this EA-induced descending adrenergic analgesic system.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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Figure 1. Effects of EA application (1 msec pulse, 2 mA, for 30 min - the EA period is indicated by hatched box) on pain behaviors in rats one day after ankle sprain. Pain levels were determined by measuring the weight bearing forces on the affected limb. A: Point specificity of EA-induced analgesic effect. The effect of EA (with 10 Hz) applied to the contralateral SI-6 acupoint was compared to that of the ipsilateral ST-36 acupoint. Only the EA at SI-6 was effective in producing analgesic effect on ankle sprain pain. B: Frequency dependency of EA-induced analgesic effect. EA with variable frequencies was applied to the SI-6 point. EA with 10 Hz stimulation produced a more powerful analgesic effect than either 2 or 100 Hz stimulation. C: The effect of phentolamine (PE; 5 mg/kg, i.p.) and naltrexone (Nal; 10 mg/kg, i.p.) on EA-induced analgesia. Drugs were administered 30 min before EA application. An α-adrenoceptor antagonist, phentolamine, but not the opioid receptor antagonist, naltrexone, was effective in blocking the EA-induced analgesic effect on ankle sprain pain. Data are presented as % changes in weight bearing forces (mean ± S.E) using the pre-EA (-30 m) baseline value as 0. *, values significantly different from the control group (No EA in A & B, EA in C).

Figure 2. Changes in the dorsal horn neuron responses to plantarflexion of the foot before and after EA in normal (n=6) and ankle sprained (n=6) rats. A & B: Examples of recorded activity evoked by plantarflexion movement (lower traces show changes of plantarflexion angle). The summary data of 6 normal and 6 ankle sprained rats are shown in C & D. s: second; m: minute; *, values significantly different from the normal group; +, values significantly different from the pre-EA value (-30 min).
Figure 3. Changes in the dorsal horn neuron responses to inversion of the foot before and after EA in normal (n=5) and ankle sprained (n=5) rats. A & B: Examples of recorded activity evoked by inversion movement (lower traces show changes of inversion angle). The summary data of 5 normal and 5 ankle sprained rats are shown in C & D. s: second; m: minute; *, values significantly different from the normal group; +, values significantly different from the pre-EA value (-30 min).

Figure 4. Changes in the dorsal horn neuron responses to compression applied to the ankle joint before and after EA in normal (n=6) and ankle sprained (n=8) rats. A & B: Examples of recorded activity evoked by compression applied to the ankle joint (lower traces show changes in compression force). The summary data of 6 normal and 8 ankle sprained rats are shown in C & D. s: second; m: minute; *, values significantly different from the normal group; +, values significantly different from the pre-EA value (-30 min).

Figure 5. Effects of pretreatment of phentolamine (PE, 5 mg/kg, i.p.) on EA-induced inhibition of dorsal horn neuron activity in ankle sprained rats. A & B: Examples of dorsal horn neuron responses to inversion of the foot before (-60 min and -30 min), during (-15 min) and after (0 min and 60 min) the EA stimulation without (A) and with (B) phentolamine pretreatment. C: Changes of the averaged mean responses from 11 neurons (4 plantarflexion, 3 inversion, and 4 compression responsive neurons combined); D: Changes of the averaged after-discharges. Arrows indicate the time of PE injection on 7 neurons (filled circle) but not 4 neurons (open circle). Hatched box represents the duration of EA application. s: second; m: minute; *: values
significantly different from the control (EA) group; +: values significantly different from the pre-EA value (at -60 m).
Fig. 1. Kim et al.,
Fig. 2. Kim et al.,

**Mean Response**

- Normal (n=6)
- Sprain (n=6)

**Changes in Response (%)**

**After-Discharges**

- Normal (n=6)
- Sprain (n=6)

**Changes in Rate (Imp/s)**

- Normal (n=6)
- Sprain (n=6)

**Time after EA (min)**

<table>
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<th>Time after EA (min)</th>
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<th>0</th>
<th>-10</th>
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<td>100°</td>
<td>20 imp/s</td>
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<tr>
<td>Ankle sprained rat</td>
<td>100°</td>
<td>20 imp/s</td>
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</tr>
</tbody>
</table>
Fig. 3. Kim et al.,

A  Normal rat

B  Ankle sprained rat

C  Mean Response

- - Normal (n=5)
- Sprain (n=5)

D  After-Discharges

- - Normal (n=5)
- Sprain (n=5)
Fig. 4. Kim et al.,

A Normal rat

Time after EA (min)

Changes in Rate (Imp/s)

B Ankle sprained rat

Changes in Response (%)

CA

D After-Discharges

Mean Response

Changes in Rate (Imp/s)

-30 -15 0 15 30 60

-20 -10 0 10 20

-30 -15 0 15 30 60

-20 -10 0 10 20

Normal (n=6)
Sprain (n=8)

Normal (n=6)
Sprain (n=8)

1,000 g
20 imp/s

20 imp/s
Figure 5. Kim et al.,