The Antipruritic Effect of Acupuncture on Serotonin-Evoked Itch in Rats

Jae-Bok Han M.D., O.M.D., Ph.D., Instructor
Chan Woo Kim O.M.D., M.A., Researcher
Boram Sun O.M.D., Assistant
Dept. of East-West Medicine, Graduate School, Kyung Hee University.
Sun Kwang Kim O.M.D., Ph.D.
Assistant, Dept. of Physiology, College of Oriental Medicine, Kyung Hee University.
Min Goo Lee M.D., Ph.D.
Prof., Dept. of Physiology, College of Medicine, Korea University.
Dong Suk Park O.M.D., Ph.D.
Prof., Dept. of Acupuncture and Moxibustion, College of Oriental Medicine, Kyung Hee University.
Byung-II Min M.D., O.M.D., Ph.D.
Prof. & Chairman, Dept. of East-West Medicine, Graduate School; Department of Physiology, College of Medicine, Kyung Hee University.

Correspondence: Dr. Byung-II Min, Department of Physiology, College of Medicine, Kyung Hee University, #1 Hoegi-Dong, Dongdaemoon-Gu, Seoul, 130-701, South Korea. Tel: +82-2-961-0286; Fax: +82-2-964-2195; E-mail: mbi@khu.ac.kr

(Received July 8, 2008; Accepted with revisions September 5, 2008 )

ABSTRACT:
The antipruritic effect of acupuncture was studied using a rat model of hindlimb scratching. After acupuncture or electroacupuncture (EA), which was conducted for 30 min, itch-associated behavior was induced by an intradermal injection of 2% serotonin (20μl) into the rostral back, and then numbers of scratching bouts were counted for 60 min. During the first experiment, acupuncture stimulations were applied to several different points. However acupuncture significantly reduced numbers of scratchings only when applied to cervical dermatomes. In the second experiment, plain acupuncture, or 2Hz, or 120Hz EA were applied to acupoints LI 11 and LI 4, at which acupuncture stimulation produced the greatest antipruritic effect in the 1st experiment, and as serotonin was administered in the same manner described for the 1st experiment. Results showed that 2Hz EA stimulation tended to increase pruritic bouts by approximately 18% versus the animals treated with plain acupuncture, whereas 120Hz EA stimulation tended to decrease pruritic bouts by approximately 39% compared with animals subjected to plain acupuncture. When nor-binaltorphimine (a κ-opioid receptor antagonist) was pretreated to elucidate the relation between κ-opioid receptor and the antipruritic effect of 120Hz EA, it was found to markedly inhibit the antipruritic effect of
120Hz EA. These results suggest that acupuncture and EA stimulation are effective treatments for pruritus if administered to dermatomes corresponding to affected sites or to adjacent dermatomes and that this effect is due to the antipruritic effect of κ-opioid receptor activation maximally induced by high-frequency EA stimulation.

Key words: Acupuncture; Electroacupuncture; Opioid receptors; Dermatome; Pruritus; Serotonin

1. Introduction

Itching, or pruritus, is a sensation that provokes a desire to scratch, and is the most common symptom of cutaneous diseases, such as atopic dermatitis, contact dermatitis and urticaria. Furthermore, it accompanies several systemic disorders, such as chronic renal failure and biliary cirrhosis. The underlying mechanisms of pruritus are unclear, but when a subset of specialized C-fibers, that originate superficially in the skin are stimulated by a pruritogen, impulses are conveyed to the dorsal horn of the spinal cord, spinothalamic tract, thalamus, and on to the somatosensory cortex. These C-fibers are anatomically identical to those associated with the mediation of pain, but are functionally distinct. The C-fibers that mediate the itching sensation comprise about 5% of the afferent C-fibers in human skin nerves, and respond to histamine and other pruritogens, but are insensitive to mechanical stimuli [1]. Itching and pain are clearly distinct sensations that originate in the peripheral and central nervous systems, but nevertheless, the peripheral and central sensitizations of pain and itching exhibit striking similarities. In one study, repetitive noxious heat and scratching were found to inhibit pruritus [2]. Thus, indicating that itching can be reduced by painful stimuli, and conversely suggesting that analgesia might reduce this inhibition and thus enhance itching [3].

Acupuncture has long been used in the Far East to treat various diseases, including pain, with few side effects, and currently is being viewed as a new alternative method of treatment in the West [4,5,6]. Studies on the mechanism of electroacupuncture (EA)-induced analgesia have revealed the roles of endogenous opioid peptides in the CNS, and other studies have demonstrated that different types of neuropeptides are released by EA [7,8,9]. Moreover, several studies have showed that low-frequency EA stimulation increases endomorphin, β-endorphin, and enkephalin levels, and that high-frequency EA stimulation increased dynorphin levels [9]. Whereas endomorphin is considered a pure μ-opioid receptor agonist [10] and dynorphin a relatively pure κ-opioid agonist [11], enkephalin and β-endorphin are mixed μ and δ opioid receptor agonists.

Table 1. The Relationships between Electroacupuncture & Endogenous Opioids / Endogenous Opioids & Their Receptors / Opioid Receptors & Itch / and Opioid Receptor Antagonists.

<table>
<thead>
<tr>
<th>Electroacupuncture</th>
<th>Endogenous Opioids</th>
<th>Receptors</th>
<th>Sensation</th>
<th>Antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Frequency</td>
<td>Endomorphin</td>
<td>μ</td>
<td>Pruritic</td>
<td>Naloxone</td>
</tr>
<tr>
<td></td>
<td>Enkephalin</td>
<td>μ</td>
<td></td>
<td>Naltrindole</td>
</tr>
<tr>
<td></td>
<td>β-endorphin</td>
<td>δ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Frequency</td>
<td>Dynorphin</td>
<td>κ</td>
<td>Antipruritic</td>
<td>Nor-binaltorphimine</td>
</tr>
</tbody>
</table>
μ- and κ-opioid receptors are important for skin homeostasis, epidermal nerve fiber regulation, and in the pathophysiology of pruritus [12]. Several observations indicate that central μ- and κ-opioid receptors are involved in the processing and regulation of the itch sensation [13,14,15]. Moreover, generalized pruritus may be induced by an imbalance between the μ- and κ-opioid systems. Activation of μ-opioid receptor stimulates itch perception, whereas activation of κ-opioid receptor inhibits the effects of μ-opioid receptor both centrally and peripherally [16,17]. Based on the above findings, we hypothesized that the activation of μ-opioid receptor by low-frequency EA stimulation aggravates pruritus, and furthermore, that the activation of κ-opioid receptors by high-frequency EA stimulation inhibits pruritus.

Several animal models of pruritus have been established, and of these models rodent hindlimb scratching behavior is increasingly being used as an itch model [18]. Furthermore, comparative studies on eight putative pruritogens (histamine, compound 48/80, kallikrein, trypsin, papain, substance P, serotonin and platelet activating factor) concluded that serotonin (5-HT) induces excessive scratching, whereas the other seven are either weak or inactive when those were injected intradermally into the rostral back of rats in order to establish an animal model of peripherally elicited pruritus [19,20,21].

It is known that acupuncture has an antipruritic effect and that this effect may be related to a spinal segment-associated inhibitory mechanism [22]. In the first experiment, plain acupuncture was applied to different points in several dermatomes including the same and adjacent dermatomes to 5-HT injection sites to identify whether the mechanism of acupuncture involves spinal segment-associated inhibition in a 5-HT-induced rat model of pruritus. In the present study, because 5-HT solution (2% and 20μl) was injected once intradermally into the rostral back, some adjacent dermatomes were also affected. Thus, the intrasegmental treatment administered during the present study probably affected the targeted dermatome and adjacent dermatomes. In the second experiment, the effects of low- and high-frequency EA on scratching behavior were compared using the acupuncture points found to be most antipruritic in the 1st experiment. To elucidate the relation between κ-opioid receptor and the antipruritic effect of high-frequency EA, an additional experiment was conducted involving the subcutaneous injection of a κ-opioid receptor antagonist.

2. Materials and Methods

2.1. Subjects
Male Sprague-Dawley rats weighing 250-350g were used in the experiment. Animals were housed in a room under controlled conditions of 25±2°C, lights on 08:00-20:00 h. Food and water were made freely available. Hair was clipped from 5-HT injection sites one day prior to injection.

Experimental Procedure

[Diagram showing time points and activities: 0 min, 30 min, 90 min, Acupuncture Stimulation, Counting the Scratching Bouts, Intradermal Injection of 5-HT Solution]
During the 1st experiment, animals were allocated to eight groups (N=9/group), and treated as follows: i) intradermal (ID) saline 20\(\mu \text{l}\) injection into the rostral back, ii) ID 5-HT 20\(\mu \text{l}\) injection into the rostral back, iii) acupuncture at unilateral Zusanli [ST 36] and Sangyingiao [SP 6] followed by ID 5-HT, iv) acupuncture at two non-acupoints in the unilateral buttock + ID 5-HT, v) acupuncture at two points over the 5-HT injection site + ID 5-HT, vi) acupuncture at unilateral Quchi [LI 11] and Hegu [LI 4] + ID 5-HT, vii) acupuncture at two non-acupoints on unilateral shoulder + ID 5-HT, and viii) acupuncture at back non-acupoints remote from the 5-HT injection site + ID 5-HT. ST 36, SP 6, LI 4, LI 11 and the itchy points (pruritic region) were selected because they are among the most commonly used points to treat pruritic disorders in Eastern medicine. Generally, acupuncture at ST 36, SP 6, LI 4 or LI 11 can easily produce strong De-qi sensation in human.

The above preliminary experiment showed that acupuncture at the LI 11 and LI 4 points had greatest antipruritic effect, and thus, these points were used in the 2nd experiment to compare the effects of plain acupuncture, and low- and high-frequency EA. Rats were allocated to the following five groups and treated as follows: i) ID saline (n=9), ii) ID 5-HT (n=10), iii) plain acupuncture followed by ID 5-HT (n=9), iv) 2Hz EA stimulation + ID 5-HT (n=8), v) 120Hz EA stimulation + ID 5-HT (n=8). In the 2nd experiment, the LI 11 and LI 4 acupoints were treated unilaterally because these treatments had the greatest antipruritic effects in the 1st experiment (Fig. 3).

To elucidate the relation between \(\kappa\)-opioid receptor and the antipruritic effect of high-frequency EA, the following two groups were added to the 2nd experiment: i) 120Hz EA stimulation + ID 5-HT, 24 h after a subcutaneous (SC) nor-binaltorphimine 10mg/kg injection in the caudal back.
A
tPRUR^IC EFFECT OF ACUPUNCTURE ON SEROTONIN-EVOKED fTCH IN RATS

(n=6), and ii) ID 5-HT, 24 h after a SC nor-binaltorphimine 10mg/kg injection in caudal back
(n=6).

2.2. Drugs
Serotonin hydrochloride (5-HT; Sigma) was dissolved in sterile physiological saline (0.9% NaCl)
at a concentration of 2% (94 mM) and 20µl was injected intradermally. This concentration was
selected because it had been reported to elicit the greatest number of scratching bouts (Jinks and
Carstens, 2002), and the 20µl volume was selected because it too showed greatest pruritogenic
effect in a preliminary study, in which we examined the effects of injection volumes of 10, 20,
30, 40µl (data, not shown). Nor-binaltorphimine dihydrochloride (nBT, Tocris) was dissolved in
physiological saline at a concentration of 1% and 0.1ml/100g body weight (10mg/kg) was SC
injected into the caudal back, 24 h before EA stimulation [16].

2.3. Acupuncture and EA stimulation
Rats were restrained in a holder and stainless-steel needles of 0.40mm diameter were inserted into
the target points in each group to a depth of 5mm and were left in place for 30 min. For EA, train-
pulses (2 or 120Hz) were applied to the needles for 30 min. The electrical current was carefully
controlled such that rats were comfortable during the EA stimulation. The rats in the control
group were also restrained in the same holder for the same length of time until the ID 5-HT
injection.

2.4. Intradermal injection of 5-HT and scratching behavior
After the 30 min period of acupuncture or EA stimulation, 5-HT solution was injected ID into the
rostral back. In the group that was treated acupuncture stimulation over the 5-HT injection site,
the 5-HT solution was injected into the skin between the two needles. After needles were
removed, rats were immediately placed into an observation chamber and videotaped from above
using a digital video camcorder for 60 min; no experimenter was allowed in the observation
room during this period. As described previously [18], one or more scratching movements by a
hind paw was defined as a scratching bout, which ended when the rat either licked its hind paw

Fig. 2: Typical waveform of pulses from the pulse generator. Axes were adjusted to accommodate waveform: divisions = 1V,
60µs for 2Hz pulse, and 1V, 20µs for 120Hz pulse. Asymmetric biphasic square waves are alternating in polarity to make
net dc current zero so as not to damage any tissue [24]. The electrostimulator has independent two frequency controls with
different pulse frequency range, and pulse width is 150 µs in low frequency range, and 50 µs in high frequency range. Voltage
was controlled between 3V and 6V such that rats were comfortable and do not squeak but weak muscle twitches were
induced and continued during 30 min.
or placed its hind paw back on the floor. Each bout consisted of rapid back-and-forth movement of the hind paw across the rostral back.

2.5. Counting and statistical analysis
The total number of scratching bouts during the 60-min observation period was counted for each animal. Statistical comparisons were made using the unpaired t-test, or one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison. P values of <0.05 were considered significant. Data are presented as means ± SEM.

3. Results
Acupuncture stimulation to a hindlimb or caudal back did not significantly influence the number of scratching bouts evoked by an intradermal injection of 5-HT into the rostral back. However, stimulation of the forelimb or the 5-HT injection site significantly decreased scratching bout numbers (Fig. 3), which implies that the spinal segment [25] may be related to the mechanism underlying the antipruritic effect of acupuncture.

Effect of acupuncture stimulation at several different acupoints on scratching behavior induced by 5-HT in rats.

![Graph showing the effect of acupuncture stimulation at several different acupoints on scratching behavior induced by 5-HT in rats.](image)

**Fig. 3:** Rats were given acupuncture (Ac.) stimuli at several different acupoints (Ap.) or were just restrained in a holder for 30 min. Intradermal 5-HT injection into the rostral back followed, and numbers of hind paw scratching bouts were counted over a 60-min observation period. Each value represents the mean ± SEM (n=9). *p<0.05, **p<0.01, ***p<0.001 versus the 5-HT rostral back group.
Acupuncture at LI 4 and LI 11 was selected to compare the antipruritic effects of plain acupuncture, and low- and high-frequency EA on 5-HT evoked pruritus at the rostral back.

**Effects of plain acupuncture stimulation and of low- or high-frequency electroacupuncture stimulation to LI4 and LI11 on scratching behavior induced by 5-HT in rats.**

![Graph showing scratching bouts induced by 5-HT](image)

**Fig. 4:** Rats were divided into the following groups: i) ID saline injection (20μl) into the rostral back, ii) ID 5-HT injection (20μl) into the rostral back, iii) Acupuncture (AT) at LI 11 and LI 4+i, iv) 2Hz EA at LI 11 and LI 4+i, v) 120Hz EA at LI 11 and LI 4+i. Numbers of hindlimb scratching bouts were counted over a 60-min observation period. Data are presented as means ± SEM. * p<0.05, ** p<0.01.

In the 1st and 2nd experiment, treatment at LI 11 and LI 4 significantly decreased scratching bout numbers in 5-HT treated rats. Furthermore, it is considered that spinal segments are probably related to the mechanism underlying the antipruritic effect of acupuncture [22,25]. The aim of the 2nd experiment was to compare the effects of plain acupuncture and 2Hz and 120Hz EA stimulation in terms of their therapeutic effectiveness. Although comparisons between the effects of plain acupuncture and 2Hz and 120Hz EA stimulation revealed no statistical differences, these results showed an interesting tendency, namely that 2Hz EA stimulation slightly counteracted the antipruritic effect of acupuncture, i.e., 2Hz EA stimulation increased scratching bouts by approximately 18% versus the plain acupuncture group. On the other hand, 120Hz EA stimulation tended to augment the antipruritic effect of acupuncture, i.e., 120Hz EA stimulation decreased scratching bouts by approximately 39% versus the plain acupuncture group (Fig. 4).

To elucidate the relation between κ-opioid receptor and the antipruritic effect of 120Hz EA...
stimulation, nBT (a κ-opioid receptor antagonist) was pretreated 24 hr before the 120 Hz EA stimulation, and was found to markedly inhibit the antipruritic effect of 120 Hz EA (Fig. 5).

**Effects of a subcutaneous injection of nor-binaltorphimine on anti-pruritic effect of high-frequency EA.**

![Graph showing the effect of subcutaneous injection of nor-binaltorphimine on anti-pruritic effect of high-frequency EA.]

Fig. 5: Two groups were added to the 2nd experiment to elucidate the relation between κ-opioid receptor and the antipruritic effect of high-frequency EA: i) 120 Hz EA at LI 11 and LI 4 for 30 min + ID 5-HT 20 μl injection into the rostral back, 24 h after a subcutaneous (SC) nor-binaltorphimine 10 mg/kg injection in the caudal back, and ii) ID 5-HT 20 μl injection into the rostral back, 24 h after a subcutaneous (SC) nor-binaltorphimine 10 mg/kg injection in caudal back. Numbers of hindlimb scratching bouts were counted over a 60-min observation period. Data are presented as means ± SEM (n=6). * p<0.05, ** p<0.01.

4. Discussion

Acupuncture and EA stimulation have been used clinically to treat pain [26,27] and pruritus [22,28,29]. Itching and pain exhibit similar patterns of central sensitization, and knowledge of the antagonistic interaction between itching, pain, and other similar sensitization processes has major implications for antipruritic therapy [30]. The antagonistic interaction between itching and pain is already exploited in pruritus therapy, and currently research continues to focus on the identification of common analgesic and antipruritic therapeutic targets [31]. In the 1st experiment of the present study, acupuncture stimulation to the same dermatome or a nearby dermatome, but not to remote dermatomes, decreased scratching bouts. Although it is known that painful stimuli, such as, scratching and cooling, can inhibit itching [32,33], no
significant reductions in scratching bout numbers were observed in the present study when acupuncture stimulation was applied extrasegmentally. These results are consistent with those of an early study on healthy human volunteers in 1987, in whom acupuncture and EA stimulation significantly reduced subjective itching intensities when applied intrasegmentally, but had no significant effect when applied extrasegmentally [22], which suggests that the antipruritic effect of acupuncture may be related to a spinal segmental inhibitory mechanism.

The 2nd experiment showed that the releases of neuropeptides induced by low- and high-frequency EA stimulations had effects that were similar to those of agonists of μ- and κ-opioid receptors on 5-HT evoked pruritus in rats. In previous studies, it has been shown that the activation of μ-opioid receptor by morphine (a μ-opioid receptor agonist) induces pruritic behavior [34], whereas naltrexone or naloxone (both μ-opioid receptor antagonists) inhibited this behavior [14,35]. It has also been shown that the scratching induced by compound 48/80 is suppressed by U-50,488H (a κ-opioid receptor agonist), but exacerbated by nor-binaltorphimine (a κ-opioid receptor antagonist) [36]. Butorphanol (a κ-agonist and μ-antagonist) was reported to effectively treat patients with chronic, severe intractable pruritus due to inflammatory skin or systemic disease [37]. It was also found that increases in endomorphin, β-endorphin, and enkephalin by low-frequency EA stimulation activate mainly μ-opioid receptor, and that increases in dynorphin induced by high-frequency EA stimulation activate κ-opioid receptor [38]. Thus, we expected that the activation of μ-opioid receptor by low-frequency EA stimulation would aggravate pruritus, and conversely, that the activation of κ-opioid receptor by high-frequency EA stimulation would inhibit pruritus.

In the 2nd experiment, comparisons of plain acupuncture stimulation, and 2Hz and 120Hz EA stimulations at LI 11 and LI 4, revealed no statistical differences, but showed an interesting tendency (Fig. 4). It was found during the 1st experiment that plain acupuncture stimulations on the same dermatome or dermatome adjacent to pruritogen injection sites have significant antipruritic effects (Fig. 3). The 2Hz and 120Hz EA stimulation groups were stimulated at the same acupoints of LI 11 and LI 4 at the same depth, and for the same time, and thus the only difference was that of frequency. Nevertheless, the difference between the two was marked. The mean number of scratching bouts in the 2Hz group (57.13) was significantly greater than in the 120Hz EA group (29.75). Demonstrating that frequency of EA stimulation importantly determines the antipruritic effect of EA.

Our additional experiments with subcutaneous nBT (a κ-opioid receptor antagonist) showed that the antipruritic effect of high-frequency EA is related to κ-opioid receptor. This administration of nBT inhibited the antipruritic effect of high-frequency EA by antagonizing the activation of κ-opioid receptor.

5. Conclusion

Acupuncture was found to reduce scratching behavior when applied to points in the same dermatome or to a dermatome adjacent to a pruritogen injection site in rats. The activation of μ-opioid receptor by low-frequency EA stimulation slightly counteracted the antipruritic effect of plain acupuncture, whereas the activation of κ-opioid receptor by high-frequency EA stimulation augmented the effect of plain acupuncture. Thus, our findings suggest that the effectiveness of acupuncture as treatment for pruritus is enhanced when high-frequency EA stimulation is performed in the dermatomes of affected sites or in adjacent dermatomes. Further studies on the interaction between pain and itching, and on the antipruritic mechanisms of acupuncture, are required.
References


