

Opioidergic orofacial antinociception induced by electroacupuncture at acupoint St36

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The participation of opioids in the antinociceptive effect of electroacupuncture was evaluated in terms of nociception produced by thermal stimuli applied to the face of male Wistar rats, weighing 180-230 g. Electrical stimulation (bipolar and asymmetric square wave with 0.5 mA intensity for 20 min) of acupoint St36, located in the anterior tibial muscle 10 mm distal to the knee joint, induced antinociception in the present model, which was maintained for 150 min. Acupoint LI4, located in the junction of the first and second metacarpal bones, did not achieve antinociception at any frequency studied (5 Hz: 1.7 ± 0.1 ; 30 Hz: 1.8 ± 0.1 ; 100 Hz: 1.7 ± 0.1 vs 1.4 ± 0.2). The antinociception obtained by stimulation of acupoint St36 was only achieved when high frequency 100 Hz (3.0 ± 0.2 vs 1.0 ± 0.1) was used, and not with 5 or 30 Hz (1.2 ± 0.2 and 0.7 ± 0.1 vs 1.0 ± 0.1). The antinociceptive effect of acupuncture occurred by opioid pathway activation, since naloxone (1 and 2 mg/kg, subcutaneously) antagonized it (1.8 ± 0.2 and 1.7 ± 0.2 vs 3.0 ± 0.1).

Key words: Acupuncture; St36 acupoint; Opioid; Orofacial antinociception

Research supported by CAPES and CNPq. Publication of the paper supported by FAPEMIG.

Received February 1, 2007. Accepted April 10, 2008

Introduction

Acupuncture, a technique of traditional Chinese medicine, originated in China more than 2500 years (1). Its philosophy conceives disease as an imbalance of the circulation of energy, denominated *Qi*, which in excess, deficiency or stagnation can lead to disease and the restoration of this circulation leads to harmony and the treatment of disease.

A number of studies have been carried out since the Ji-Sheng group published data demonstrating the analgesic effect of acupuncture in humans (2) to elucidate the physiological mechanisms by which acupuncture produces its effects. Acupuncture analgesia was reported to occur via the mobilization of endogenous opioids in the spinal cord (3,4). They also reported that opioid mobilization varied

with frequency. High and low stimulation frequencies liberate different kinds of opioids (5-7). Opioid release also seems to occur in the brain, as demonstrated by the *icv* injection of naloxone, a non-selective opioid receptor antagonist, which can block the antinociception induced by electroacupuncture (4).

The efficacy of acupuncture analgesia is well documented, and some clinical trials have demonstrated that acupuncture diminishes the need for post-operative use of non-steroidal (8) and steroidal analgesics (9). Others have shown that acupuncture is effective in treating migraine crisis (10) and orofacial pain (11) without the use of pharmacological agents. However, less is known about its action on trigeminal pain. There are few studies on humans concerning orofacial pain and even fewer controlled laboratory and animal studies (12,13).

The objectives of the present study were: a) to determine the efficacy of acupuncture antinociception in a model of orofacial nociception in rats using points recognized to be analgesic, St36 (14-18) and LI4 (19-22), and b) to assess if this effect occurs via endogenous opioid mobilization.

Material and Methods

Animals

Male Wistar rats, weighing 180-230 g, were used. Two days before the tests, they were housed in a room with controlled temperature ($23 \pm 1^\circ\text{C}$) and a 12-h light/dark cycle (lights on at 7 pm) with food and water *ad libitum*. The experiments were conducted during the light part of the cycle. All procedures were performed in accordance with protocols approved by the Ethics Committee of the Federal University of Minas Gerais, which essentially follow the ethical guidelines of the International Association for the Study of Pain in Conscious Animals (23).

Algesimetric method

The rat was hand-restricted for up to 10 s with the right side of the face (vibrissa region) laid across an NiCr wire coil at room temperature ($23 \pm 2^\circ\text{C}$). This area was used to stimulate the nociceptive endings of the maxillary branch of the trigeminal nerve (24-26). The coil temperature was then increased by an electric current until a head withdrawal reflex was obtained. In order to adapt to the algesimetric test, the rats were familiarized with the apparatus twice, 1 day before the experiments.

The intensity of the heat was adjusted so that the baseline latencies were between 3 and 4 s. The cut-off time

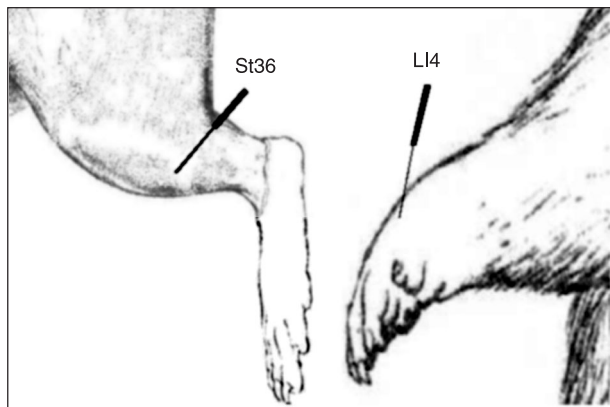


Figure 1. Placement of the acupuncture needles in the rat. Acupoint St36 is located in the anterior tibial muscle, 10 mm distal to the knee joint. Acupoint LI4 is located in the junction of the first and second metacarpal bones.

was established at 8 s to reduce the possibility of tissue damage.

Immobilization and electroacupuncture stimulation

Rats were immobilized in a plastic cylinder that allowed access to all four limbs for the application of acupuncture needles (17,18). This apparatus permits acupuncture stimulation in awake rats. It was used for two reasons: 1) to avoid the interference of anesthetic procedures, and 2) to make the procedure as close as possible to clinical practice. To minimize stress-induced antinociception, the rats were familiarized with the immobilization apparatus for 30 min each day for 2 days prior to the experiments.

Stainless steel needles (7 x 0.17 mm) were inserted bilaterally into acupoint St36, located in the anterior tibial muscle, 10 mm distal to the knee joint, or into acupoint LI4, located in the junction of the first and second metacarpal bones (Figure 1). The needles were connected to the output of an electronic pulse generator (Sikuro DS100, Brazil), which produces a bipolar and asymmetric square wave. The frequencies tested were 5, 30, and 100 Hz, and the duration of stimulation was 20 min for all frequencies. Stimulus intensity was maintained subthreshold, set at 0.5 mA intensity, just below a detectable muscle twitch or rat vocalization, in order to mimic the intensity used in clinical practice as closely as possible.

Drugs

Naloxone hydrochloride (Sigma, USA), a non-selective opioid receptor antagonist, was diluted in 0.9% saline, and injected subcutaneously (1 mL/kg) into the dorsal nuchal area to obtain a systemic distribution of the drug. Naloxone was administered 10 min before acupuncture stimulation.

Experimental design

The rats were randomly distributed into two groups to evaluate the efficacy of acupoints St36 (group 1) and LI4 (group 2) in promoting orofacial antinociception. The baseline latency was measured immediately before the electroacupuncture stimulation, which was bipolar and asymmetric square wave with 0.5 mA intensity for 20 min. Both groups were tested at 5, 30, and 100 Hz. A control group received acupuncture, but without any stimulation. The first withdrawal latency measurement was taken immediately after terminating electroacupuncture stimulation. Subsequent measurements were made every 15 min during the first hour and every 30 min during the second and third hours.

The baseline latency was measured immediately before drug injection. Naloxone (0.5, 1, 2 mg/kg) was injected

10 min before the onset of electroacupuncture stimulation. The control groups received the same volume of saline.

Statistical analysis

The results are reported as the mean value of 3 measurements per rat, or as the mean area under the curve \pm SEM for 5 rats in each group. Statistical analysis was carried out with ANOVA followed by Bonferroni's test. The results were considered to be significant when P values were less than 0.05, using the Prisma software (version 3.0).

Results

Acupoint St36

The stimulation of acupoint St36 produced a significant increase in the nociceptive threshold when 100 Hz frequency was used. This effect began immediately after terminating electroacupuncture stimulation, peaked at 30 min and remained statistically significant for 150 min. The

other frequencies tested (5 and 30 Hz) did not induce an antinociceptive effect at any of the times tested (Figure 2A).

Acupoint LI4

Stimulation of acupoint LI4 did not increase the withdrawal threshold in rats that were submitted to heat. All frequencies applied to this acupoint (5, 30, and 100 Hz) failed to promote the antinociceptive effect observed for acupoint St36 (Figure 2B). This acupoint became efficient when the intensity of stimulation was increased 4-fold (Figure 2C).

Effect of naloxone

Administration of naloxone (1 or 2 mg/kg, subcutaneously) produced an antagonistic effect on the antinociception induced by 100 Hz frequency stimulation applied to acupoint St36. There was a reduction in the latency of the rats to flinch and withdraw their faces from the heat source when compared with the respective control. No statistical

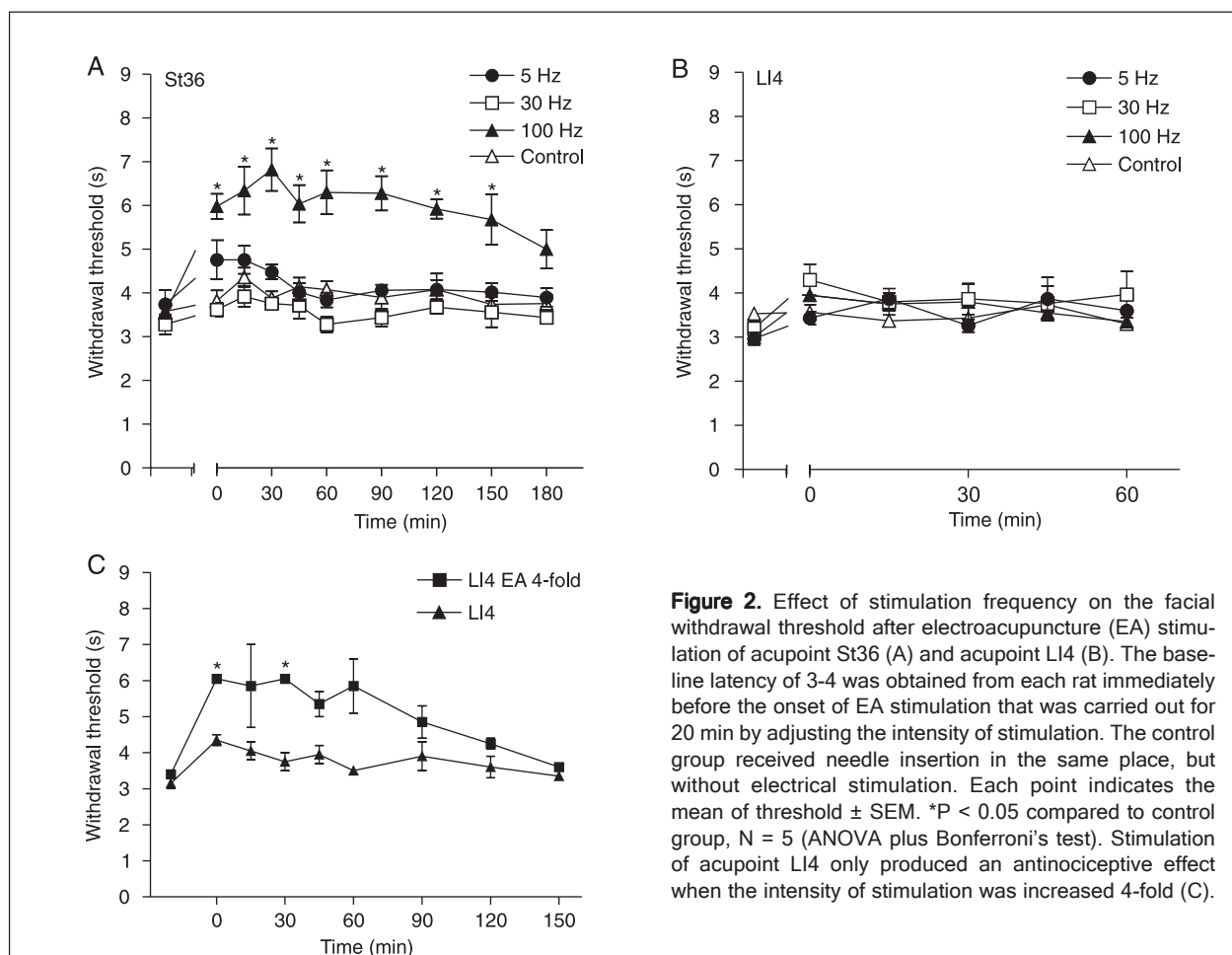


Figure 2. Effect of stimulation frequency on the facial withdrawal threshold after electroacupuncture (EA) stimulation of acupoint St36 (A) and acupoint LI4 (B). The baseline latency of 3-4 s was obtained from each rat immediately before the onset of EA stimulation that was carried out for 20 min by adjusting the intensity of stimulation. The control group received needle insertion in the same place, but without electrical stimulation. Each point indicates the mean of threshold \pm SEM. *P < 0.05 compared to control group, N = 5 (ANOVA plus Bonferroni's test). Stimulation of acupoint LI4 only produced an antinociceptive effect when the intensity of stimulation was increased 4-fold (C).

difference was found between doses of 1 and 2 mg/kg. Naloxone at 0.5 mg/kg did not antagonize acupoint St36 stimulation-induced antinociception. In addition, naloxone alone showed no effect on the latency to flinch or withdraw the face (Figure 3).

Discussion

The present data demonstrated that electroacupunc-

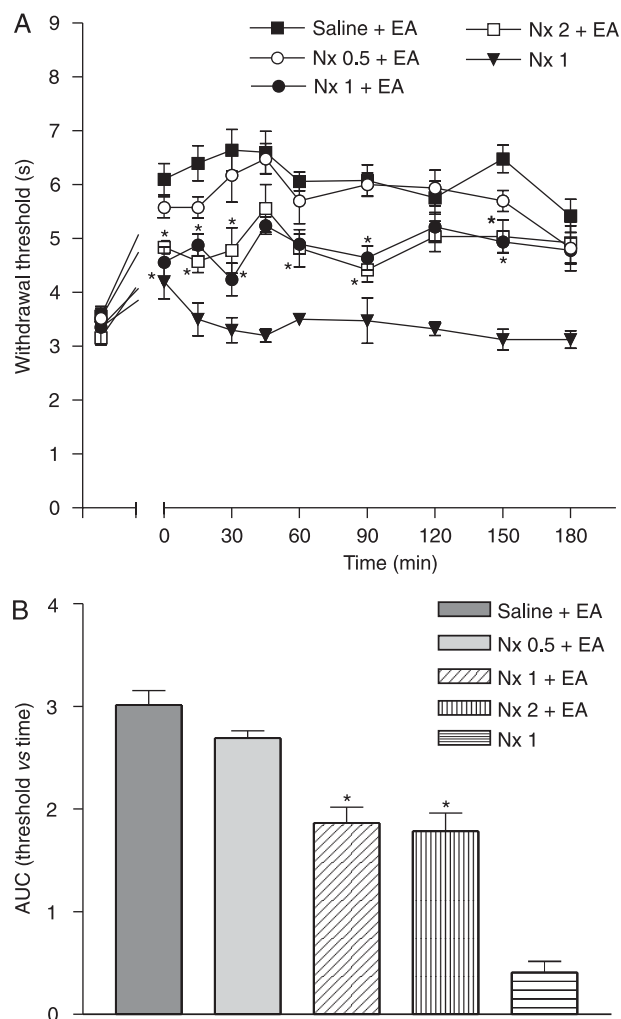


Figure 3. Effect of naloxone on antinociception in the rat induced by electroacupuncture (EA) at acupoint St36. The baseline latency was obtained immediately before drug injection as described in the legend to Figure 2. Naloxone (Nx, 0.5, 1, 2 mg/kg) was injected 10 min before the initiation of EA stimulation, which lasted 20 min. The control group received acupuncture but without any stimulation (NX 1). Data are reported as the mean of the facial threshold \pm SEM and as AUC (area under the curve), threshold vs time, in *panel B*. * $P < 0.05$ compared to control (ANOVA plus Bonferroni's tests).

ture applied in acupoint St36 is able to promote orofacial antinociception in rats, as measured by a facial modification of the "tail-flick" test. These results are similar to previous reports that used the same acupoint but not the same method to measure antinociception (27-29). However, unlike several studies that obtained antinociception at both high and low frequencies (5,6,30,31), the present results demonstrated that only 100 Hz frequency, not 5 or 30 Hz, was able to promote this effect. One possible explanation for this divergence is the intensity of stimulation, which was subthreshold in the present method in order to make our protocol as similar as possible to the clinical application of acupuncture. The majority of other studies use suprathreshold stimulation intensities, which seem to promote greater neurotransmitter mobilization and thus, induce antinociception at all frequencies tested.

Earlier studies demonstrated that stimulation of acupoint LI4 induced analgesia in humans (20,21,30-32), and antinociception in animals (33,34). However, the current results showed no similar orofacial effect of acupoint LI4 in these animals. Again, the stimulation intensity may be a significant methodological difference between the present study and other published research. With the aim to test this hypothesis, an experiment was conducted in our laboratory when the acupoint was used with suprathreshold intensities of stimulation (4-fold muscle twitch response), and in this case, this point was capable to promote orofacial antinociception (Figure 2C).

Differences in the results of studies on animals and humans when using acupoint LI4 stimulation seem to demonstrate a different activation mechanism, since LI4 in humans was efficient at subthreshold stimulation intensities, which was not demonstrable in the present work on rats. One relevant fact is that in the orofacial analgesic studies in humans the LI4 point was never used without combination with other points. In the majority of studies that demonstrated positive results, this acupoint was used in combination with local points. Consequently, an assessment of the orofacial analgesic potential of acupoint LI4 in humans without the simultaneous use of other points is required.

The present results demonstrated that needle insertion without stimulation in acupoint St36 did not produce antinociception. This is in agreement with previous research on humans (30,35). There is a lack of research on animals investigating this type of control.

The restraint apparatus would be capable of causing freezing stress behavior in rats, which could be understood as antinociception. With the aim to test this hypothesis, our group conducted an experiment that did not show the influence of stress in our experimental groups.

Thus, this hypothesis was not confirmed by our experimental data, which are in accordance with other studies that used the same restraint method (results not shown) (17,18).

Numerous studies on both animals and humans have shown that acupuncture antinociception can be blocked by naloxone (3,4), which suggests the involvement of opioid receptors. In addition, low frequencies of electroacupuncture stimulation (2 and 30 Hz) release met-enkephalin, β -endorphin and endomorphin via μ and δ receptors, while electroacupuncture antinociception induced by high frequencies (100 and 200 Hz) is mediated by dynorphin via κ

receptors (5-7). The current findings demonstrated that orofacial antinociception induced by electrical stimulation in acupoint St36 occurs by opioid pathway activation, probably by dynorphin mediation, since naloxone also blocked this effect (Figure 3B). Results reported by our group showing that opioids activate the L-arginine/nitric oxide/cGMP pathway which activates K^+ channels (36-39), and that nitric oxide is involved with acupuncture antinociception (40) suggest the hypothesis that electroacupuncture at acupoint St36 leads to nitric oxide formation, which then promotes antinociception.

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