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RESEARCH ARTICLE

100-Hz Electroacupuncture but not 2-Hz Electroacupuncture is Preemptive Against Postincision Pain in Rats

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Abstract
Preemptive analgesia involves introducing an analgesic before noxious stimulation. Electroacupuncture (EA) activates descending mechanisms that modulate nociceptive inputs into the spinal dorsal horn. This study evaluated whether preoperative EA is more effective than postoperative EA in reducing incision pain in rats. The nociceptive threshold to mechanical stimulation was utilized to examine the effects of an intraperitoneal injection of saline (0.1 mL/kg) or naloxone (1 mg/kg) on antinociception induced by a 20-minute period of 2-Hz or 100-Hz EA applied to the Zusanli (ST36) and Sanyinjiao (SP6) acupoints before surgical incision, or 10 minutes after or 100 minutes after surgical incision of the hind paw. The extent of mechanical hyperalgesia after the incision was significantly attenuated by the application of 100-Hz EA preoperatively, but not by its application at 10 minutes or 100 minutes postoperatively. By contrast, 2-Hz EA was effective against postoperative hyperalgesia when applied 10 minutes or 100 minutes after surgery but not when it was applied preoperatively. Only the effect of 2-Hz EA applied 10 minutes after surgery was sensitive to naloxone. The present study showed for the first time that 100-Hz EA, but not 2-Hz EA, exerts a nonopioidergic preemptive effect against postincision pain in rats.

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1. Introduction

Within a few postsurgical days after the generation of lesions during the surgical procedure, 50% of patients experience intense or very intense pain because of hyperalgesia and/or allodynia [1,2]. This phenomenon is accompanied by increased excitability of spinal nociceptive cells and excessive cell depolarization in response to peripheral stimulation [3]. Therefore, pretreatment for pain may prevent the changes in the central nervous system that follow persistent noxious inputs [4].

The importance of interventions initiated before a painful stimulus in reducing the development of cell hyperexcitability (i.e., preemptive analgesia), which may diminish postoperative pain, has been discussed extensively [5]. One objective of preemptive analgesia is to reduce pain resulting from mechanisms triggered by a surgical incision [6].

Electroacupuncture (EA) has been widely used for pain treatment [7]. EA-induced analgesia involves the activation of several brain structures that are implicated in pathways that descend through the dorsolateral funiculus to modulate nociceptive inputs in the spinal dorsal horn [8,9]. EA decreases C-fiber activity in normal rats after formalin injection [10]; therefore, EA may effectively produce preemptive analgesia. Taking into account the current understanding of preemptive analgesia, the present study evaluated whether preoperative EA more effectively reduces postsurgical pain, compared with postoperative EA.

2. Materials and methods

2.1. Animals

The experiments were conducted on male Wistar rats (140–160 g) and were approved by the Commission of Ethics in Animal Research in the Faculty of Medicine of Ribeirão Preto at the University of São Paulo (São Paulo, Brazil; Approval No. 078/2011). The experiments followed the guidelines of the International Association for the Study of Pain (IASP) [11].

2.2. Model of incision pain

Each animal was anesthetized with isoflurane in oxygen flow (2% for induction and 0.5% for maintenance) via a loose-fitting, cone-shaped mask. Beginning 0.5 cm from the proximal edge of the heel, a 1-cm longitudinal incision was formed through the skin and fascia of the plantar aspect of the right hind paw, as described elsewhere [12]. The skin was then sutured with two 5-0 nylon stitches.

2.3. Mechanical nociceptive threshold

To facilitate behavioral acclimation, 15–30 minutes before the beginning of the test, the rats were placed in elevated plastic cages (12 cm × 20 cm × 17 cm) containing wire-grid floors. The threshold of the response to mechanical stimulation (MS) was measured using an electronic von Frey apparatus (IITC Electronic Equipment, Woodland Hills, CA, USA), which consisted of a handheld probe unit connected to a rigid plastic tip (tip area, 0.7 mm^2). The tip was applied at an increasing force in an upward direction and continuously recorded at sites near the heel, at 1–2 mm medial to the side of the subsequent incision (i.e., baseline), or at the actual incision until the animal withdrew the stimulated paw. Each trial consisted of three applications of the tip, once every 5 seconds. The mean threshold of the three trials was considered the threshold for a particular time point.

2.3.1. EA

To minimize the stress induced by animal restraint, the procedures were performed on rats that were lightly anesthetized with isoflurane in oxygen flow (2% for induction and 0.5% for maintenance) through a loose-fitting cone-shaped mask. Stainless steel acupuncture needles (diameter, 0.3 mm; length, 30 mm) were inserted at a depth of 5 mm bilaterally into the hind legs at acupoints Zusanli (ST36) and Sanyinjiao (SP6). The stimuli were generated by a constant current pulse generator (NKL, Brusque, Santa Catarina, Brazil) and simultaneously applied for 20 minutes to both hind legs. The stimuli were set as square waves—0.5 ms in width at a frequency of 2 Hz or 100 Hz—and the intensity (140–150 μA) was increased in a stepwise manner until a muscle twitch occurred. EA was performed using a current intensity 10-fold higher than the muscle twitch threshold of each animal [13–15]. Animals allocated to the sham EA groups underwent needle insertion into the same acupoints but no stimulation with electrical current [16]. This allowed the assessment of the possibility that the simple insertion of needles significantly influences the nociceptive threshold [17].

2.4. Experimental protocol

The protocol of this study is summarized in Fig. 1. The experiments were conducted using sham EA, 2-Hz EA, or 100-Hz EA. For each EA condition, the animals were randomly assigned to one of three groups comprising six rats each. The protocol for each group is as follows:

Group 1: the baseline (BL) nociceptive threshold was measured, and saline (0.1 mL/kg) was then injected intraperitoneally. Five minutes later, the animals were anesthetized and subjected to a 20-minute period of EA. Two minutes after EA, a surgical incision was formed in the right hind paw, and anesthesia was discontinued.

Group 2: the BL nociceptive threshold to MS was measured. Twenty-two minutes after the BL measurement, the animals were anesthetized and subjected to a surgical incision of the right hind paw. Five minutes later, saline (0.1 mL/kg) was injected intraperitoneally. Five minutes after this, EA was applied for 20 minutes and anesthesia was discontinued.

Group 3: the BL nociceptive threshold was measured. Twenty-two minutes later, the animals were anesthetized and subjected to a surgical incision of the right hind paw. Anesthesia was discontinued and reinstalled 95 minutes later. Five minutes after the reinstallation of anesthesia, saline (0.1 mL/kg) was injected intraperitoneally. Five minutes after this injection, the animals were subjected to 20 minutes of EA, and anesthesia was discontinued once again.

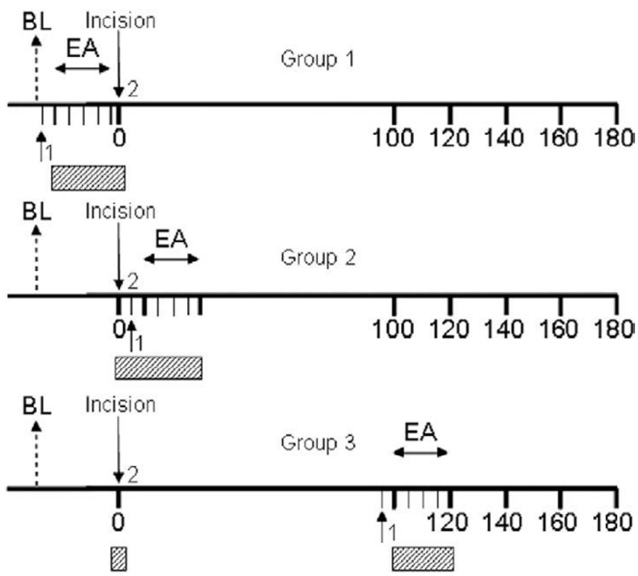

Figure 1 Summary of the protocol used in this study. The timeline shows the time points of electroacupuncture (EA; horizontal arrow), saline or naloxone injection (vertical arrow 1), and surgical incision (vertical arrow 2). The horizontal boxes represent the periods of isoflurane anesthesia for surgery and EA. The time is presented in minutes. BL = baseline; EA = electroacupuncture.

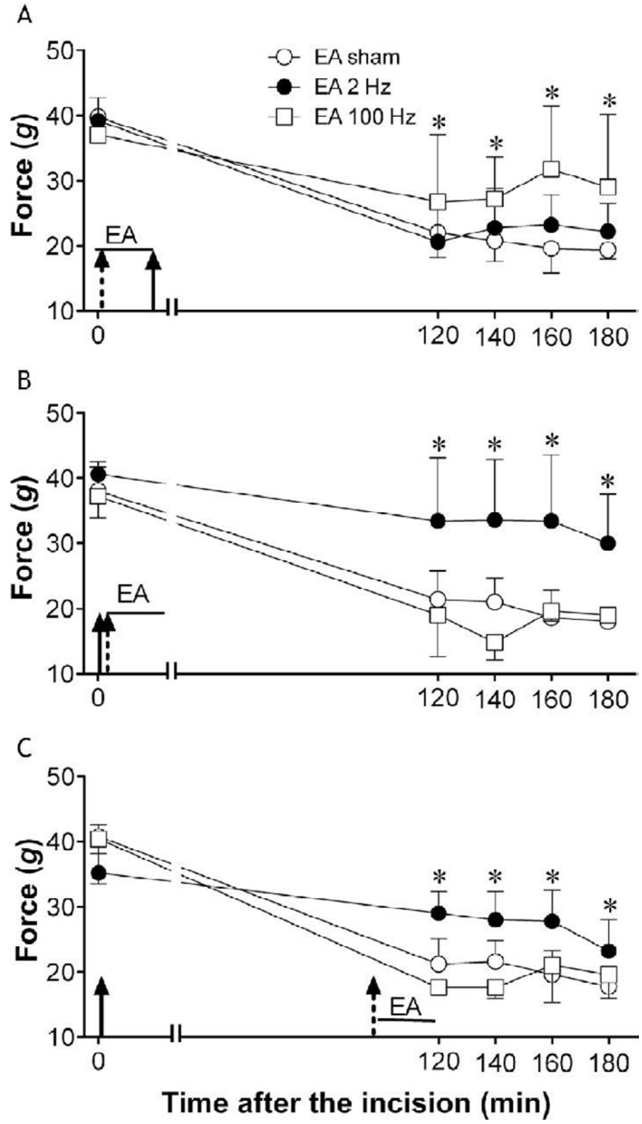

Figure 2 The effects of electroacupuncture (EA) on the nociceptive threshold to mechanical stimulation applied to the incised hind paw of rats. EA (horizontal bar) was conducted at the indicated frequencies (A) before the incision, (B) 10 minutes after the incision, or (C) 100 minutes after the incision, which is indicated by the solid arrow. Surgery and EA were conducted on animals that were anesthetized with isoflurane. Saline (0.1 mL/kg) was injected intraperitoneally at the time point indicated by the dashed arrow. The points represent the mean $\pm$ the standard deviation of six rats per group. * $p < 0.05$, compared with the sham EA group.

The threshold of the response to MS in all groups was measured at 20-minute intervals, beginning 100–180 minutes after surgery. Three additional groups of six rats were subjected to similar protocols, but naloxone (1 mg/kg), instead of saline, was administered via the intraperitoneal route. Whenever the groups of rats did not differ in their BL responses, the difference in the nociceptive thresholds between the postincision time point and BL was calculated for each animal to facilitate comparing the effects of different experimental conditions.

2.5. Statistical analysis

The differences in the nociceptive threshold between BL and each postincision measurement are reported as the mean $\pm$ the standard deviation. The control (i.e., sham EA) rats and experimental groups were compared via multivariate analysis of variance (MANOVA) [18]. The factors analyzed were group, time, and group $\times$ time interaction. In each instance of a significant group $\times$ time interaction, ANOVA was performed, followed by the Duncan test. The analysis was performed using SPSS/PC+ software, version 17.0 (SPSS Inc., Chicago, USA). The level of significance was set at $p < 0.05$ for all conditions.

3. Results

3.1. Changes in postincision pain induced by 2-Hz and 100-Hz EA

The effects of EA under different experimental conditions are shown in Fig 2. The groups did not significantly differ with respect to the BL nociceptive thresholds. Under all experimental conditions, the nociceptive threshold of the incised paw was significantly reduced 2 hours after the incision. The nociceptive threshold remained stable and below the BL level throughout the observation period in the sham EA-treated animals. The animals that were subjected to 100-Hz EA before the incision showed a difference in the nociceptive threshold between BL and 120–180 minutes after the incision that was significantly smaller than the threshold in the control animals. By contrast, the animals subjected to 2-Hz EA before the incision exhibited an insignificant difference in the nociceptive threshold, compared with the control animals, throughout the observation period (Fig. 2A). The change after the incision was significantly smaller in animals subjected to 2-Hz EA for 10 minutes (Fig. 2B) or 100 minutes (Fig. 2C) than in the control animals at 120—180 minutes after the incision. By contrast, the animals subjected to 100-Hz EA at 10 minutes or 100 minutes after the incision had an insignificant change in the nociceptive threshold, compared with the control animals throughout the observation period under the two experimental conditions (Figs. 2B and 2C). The curves presented in Fig. 2 show a significant effect of group ($F_{2,12} = 6.41$, $F_{2,12} = 7.98$, and $F_{2,12} = 12.70$ for Graphs A, B, and C, respectively; $p < 0.01$, for all conditions) and were not significantly different with regard to time ($F_{3,36} = 0.21$, $F_{3,36} = 1.19$, and $F_{3,36} = 1.94$; $p > 0.05$, for all cases) or group $\times$ time interactions ($F_{6,36} = 0.92$, $F_{6,36} = 1.90$, and $F_{6,36} = 1.77$; $p > 0.05$, for all conditions).

3.2. Changes induced by intraperitoneal naloxone on the effects of EA on postincision pain

The time course of the effects of EA under different experimental conditions is shown in Fig. 3. All rats received naloxone (1 mg/kg) intraperitoneally 5 minutes before EA. The groups also did not differ significantly with respect to the BL nociceptive thresholds. Two hours after the incision, the nociceptive threshold of the incised paw was significantly reduced under all experimental conditions. The nociceptive threshold remained stable and below the BL level throughout the observation period in the sham EA-treated animals. The difference in the nociceptive threshold was significantly smaller in animals subjected to 100-Hz EA before the incision than in the control animals at 120—180 minutes after the incision (Fig. 3A). By contrast, application of 2-Hz EA before the incision produced an insignificant difference in the nociceptive threshold, compared with the control animals during the same period (Fig. 3A). Application of 2-Hz or 100-Hz EA at 10 minutes or 100 minutes after the incision produced an insignificant difference in the nociceptive threshold, compared with the control animals (Figs. 3B and 3C, respectively). The curves in Fig. 3A indicate a significant effect of group ($F_{2,12} = 9.12$; $p < 0.01$), but the curves in Figs. 3B and 3C show no significant effects ($F_{2,12} = 0.02$ and $F_{2,12} = 11.49$, respectively; $p > 0.05$ for all conditions). The curves in Fig. 3 show no significant effect of time ($F_{3,36} = 1.12$, $F_{3,36} = 0.37$, and $F_{3,36} = 1.18$ for Graphs A, B, and C, respectively; $p > 0.05$ for all conditions) or group $\times$ time interaction ($F_{6,36} = 1.41$, $F_{6,36} = 2.48$, $F_{6,36} = 2.1$ for Graphs A, B, and C, respectively; $p > 0.05$ for both experimental conditions).

4. Discussion

All rats in this study responded to MS of the hind paw before the skin incision, and all rats responded to light MS of the same region after the incision. A surgical incision evokes spontaneous activity of nociceptive fibers [19], sensitizes spinal dorsal horn cells [20], increases the size of the peripheral receptive field [21], and increases the response of spinal-wide dynamic range neurons to MS [22]. The treatment of pain before its initiation, a procedure called preemptive analgesia, may prevent the changes in the central nervous system that follow persistent noxious input [4].

EA-induced analgesia exhibits no additional effect when applied to patients before hysterectomy [23] or knee arthroscopy [24]. Applying manual acupuncture

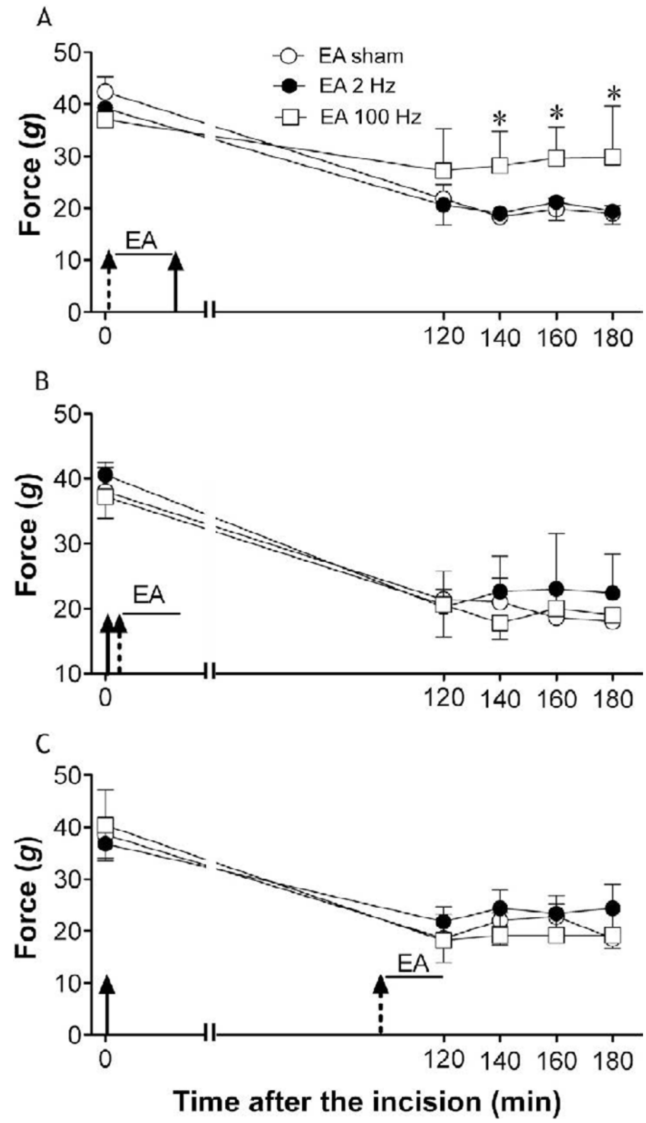

Figure 3 Changes produced by intraperitoneal injection of naloxone (dashed arrow) in the effects of electroacupuncture (EA) on the nociceptive threshold to mechanical stimulation of the incised hind paw of rats. EA (horizontal bar) was conducted at the indicated frequencies (A) before the incision, (B) 10 minutes after the incision, or (C) 100 minutes after the incision, which is indicated by a solid arrow. Surgery and EA were conducted on animals that were anesthetized with isoflurane. Naloxone (1 mg/kg) was injected at the time point indicated by the dashed arrow. The points represent the mean ± the standard deviation of six rats per group. * $p < 0.05$, compared with the sham EA group.

10 minutes before surgery increased postoperative pain and the consumption of analgesics after the surgical removal of impacted mandibular third molars [25]. However, EA reduced the need for postoperative analgesics when applied 20 minutes before lower abdominal surgery [26] or 12—18 hours before conventional heart surgery [27].

Few reports are available regarding the preemptive property of EA in animal models of pain, and all previous studies evaluated the effects of low-frequency EA against inflammatory pain [28—30]. Application of 10-Hz EA 30 minutes before intraplantar formalin injection into rats attenuated nociceptive behavior and c-Fos immunoreactivity in the lumbar spinal dorsal horn [28]. The antihyperalgesic effect of 3-Hz EA [29] or 10-Hz EA [30] applied to rats before intraplantar carrageenan administration has also been demonstrated.

The present study showed that postincision mechanical hyperalgesia was significantly attenuated by applying 100-Hz EA preoperatively, but not when applying it 10 minutes or 100 minutes postoperatively. Based on this result, 100-Hz EA appears to exert a preemptive effect against postincision mechanical hyperalgesia in rats. However, this result contrasts with a previous observation that acupuncture is effective in post-traumatic situations but is not preemptive against pain generated by knee arthroscopy [24]. The inefficacy of postoperative 100-Hz EA also contrasts with the findings of one study [31] that demonstrated that applying 100-Hz EA at 3 days after surgery was very effective against postincision mechanical hyperalgesia in rats. The different time points of EA application used in each aforementioned study may account for the discrepancies in the results of the current study.

In contrast to the preemptive effect of 100-Hz EA, 2-Hz EA was effective against postincision hyperalgesia when applied 10 minutes or 100 minutes after surgery, but not when applied before surgery. The analgesic effects of low- and high-frequency EA depend on the activation of different descending mechanisms [32]; however, both systems can modulate nociceptive inputs into the spinal dorsal horn [8]. Thus, we suspect that the preemptive effect of 100-Hz EA depends on an undetermined property of EA, aside from the exclusive activation of pain-inhibitory descending mechanisms.

Incision pain perhaps depends more on postoperative peripheral inflammation than on the central sensitization that develops during surgery [33,34]. Preemptive analgesia would occur only when the local generation of prostaglandins and leukotrienes evoked by surgical incision is pharmacologically prevented [35].

Differences in the effects of EA against surgical and inflammatory animal models of pain have been observed [29]. The application of 10-Hz EA or 100-Hz EA inhibited complete Freund's adjuvant-induced hyperalgesia of the hind paw [36—38]. However, 10-Hz EA but not 100-Hz EA significantly reduced complete Freund's adjuvant-induced hind paw edema [39]. Application of 2-Hz EA reduces inflammatory pain and the release of proinflammatory cytokines [40]. In addition, 100-Hz EA has a potent short-term inhibitory effect on hyperalgesia but little effect on inflammation, whereas 10-Hz EA exerts moderate and prolonged antihyperalgesic and anti-inflammatory effects [13,41]. Furthermore, the effects of EA on hyperalgesia and inflammation are mediated by two distinct pathways [42].

Low-frequency EA suppresses inflammatory pain in mice via sympathetic postganglionic neurons, whereas high-frequency EA suppresses pain via the sympathoadrenal medullary axis [38]. The reactions to a surgical incision involve inflammatory responses to local injury [43—45], but the model of incision pain differs from classic models of inflammatory pain [46].

The effects of preoperative 100-Hz EA and late postoperative 2-Hz EA were not affected by the previous administration of naloxone, whereas the antihyperalgesic effect of 2-Hz EA applied 10 minutes after the incision was sensitive to naloxone. Opioid receptors on nerve endings are upregulated during inflammation, and EA-induced anti-inflammatory activity is reduced after the injection of naloxone at the site of injury [29]. The application of 3-Hz EA after the administration of carrageenan activates μ-, δ-, and κ-opioid receptors on peripheral nerve terminals [47]. However, the anti-inflammatory effect of EA occurs via a nonopioidergic mechanism in the central nervous system [29,39].

The present study thus showed for the first time that 100-Hz EA, but not 2-Hz EA, exerts a preemptive effect against incision pain in rats. In addition, the preemptive effect of 100-Hz EA does not depend on the modulation of the opioid system.

Disclosure statement

The author declares to have no conflicts of interest and no financial interests related to the material of this manuscript.

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