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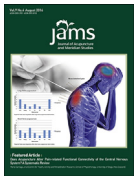

RESEARCH ARTICLE

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.