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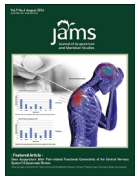

Research Article

Isoflurane and the Analgesic Effect of Acupuncture and Electroacupuncture in an Animal Model of Neuropathic Pain

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Abstract
The present study aimed to determine whether isoflurane interferes with the analgesic effects of acupuncture (Ac) and electroacupuncture (EA), using a neuropathic pain (NP) rat model. In total, 140 male Wistar rats were used; isoflurane-induced nociceptive response was evaluated using the von Frey test, serum calcium-binding protein β (S100β) levels and nerve growth factor (NGF) levels in the left sciatic nerve. The NP model was induced by chronic constriction injury of the sciatic nerve at 14 days after surgery.

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Treatment was initiated after NP induction with or without isoflurane anesthesia (20 min/day/8 days). The von Frey test was performed at baseline, 14 days postoperatively, and immediately, 24 h, and 48 h after the last treatment. Results of the nociceptive test and three-way analysis of variance were analyzed by generalized estimating equations, the Bonferroni test, followed by Student—Newman—Keuls or Fisher's least significant difference tests for comparing biochemical parameters (significance defined as $p \leq 0.05$). At baseline, no difference was noted in the nociceptive response threshold among all groups. Fourteen days after surgery, compared with other groups, NP groups showed a decreased pain threshold, confirming establishment of NP. Ac and EA enhanced the mechanical pain threshold immediately after the last session in the NP groups, without anesthesia. Isoflurane administration caused increased nociceptive threshold in all groups, and this effect persisted for 48 h after the last treatment. There was an interaction between the independent variables: pain, treatments, and anesthesia in serum S100β levels and NGF levels in the left sciatic nerve. Isoflurane enhanced the analgesic effects of Ac and EA and altered serum S100β and left sciatic nerve NGF levels in rats with NP.

1. Introduction

Acupuncture (Ac) and electroacupuncture (EA) treatments have yielded good results for alleviating neuropathic pain (NP) pain in humans and experimental animals [1,2], though the exact mechanisms of action are unknown. Owing to the practical difficulties in Ac and EA application to awake and freely moving animals, most studies have used restraint [3] or anesthesia [4]. However, anesthetization or immobilization during treatment may lead to physiological changes which could potentially affect treatment efficacy.

The evaluation of Ac and EA may be biased by restraint stress or habituation in conscious animals [5] or by the anesthetics used in sedated animals. The application of Ac or EA treatment to awake animals (insertion of a needle or manual or electric stimulation of animals) may be viewed as stressors. Restraint, shock, and fear are known to trigger stress-induced analgesia when animals are awake, as shown in models of Ac and EA analgesia [6]. Therefore, Ac and EA analgesia can be significantly reduced if concomitant stressors are not adequately controlled.

In animal studies of Ac or EA, isoflurane is the most commonly used anesthetic [4]. It is easily administered and produces the behavioral and physiological characteristics of general anesthesia without an adjunct [7]. Nevertheless, whether it can alter the analgesic response to Ac or EA treatment is unknown. Interestingly, anesthesia may influence biomarker expression. Mice exposed to isoflurane during postnatal brain development showed increased serum levels of calcium-binding protein β (S100β), a protein used as a neurodegenerative biomarker [8], though this has not been studied in adult rats.

Neurotrophins help in neuronal survival, growth, and differentiation and may also be affected by isoflurane. Nerve growth factor (NGF) is a pain-related neurotrophin that can exert pronociceptive or antinociceptive effects, depending on concentration and site of administration [9]. Chen et al. demonstrated the neuroprotective effect of EA-induced neurotrophins in an animal model of spinal cord injury [10], indicating that EA may reduce pain by neurotrophic modulation.

Based on these findings, we believe that isoflurane can potentiate the analgesic effect of Ac and EA treatments and modify neuromodulation parameters. To test this hypothesis, we evaluated the nociceptive response induced by isoflurane, the serum levels of S100β and NGF in the left sciatic nerve of Ac- or EA-treated NP rats, using the von Frey test. Concurrently, we assessed locomotor behavior to demonstrate the extent to which the animals were affected by anesthesia.

2. Materials and Methods

2.1. Animals

A total of 140 male Wistar rats (weight ≥250 g) aged 55—65 days were used in the experiment. Based on our previous studies, 140 animals were deemed to produce reliable scientific data [11, 12, 13]. Animals housed individually in polypropylene cages (49 × 34 × 16 cm) in a controlled environment (22 ± 2°C) under a standard light—dark cycle (lights-on/lights-off: 0700 h/1900 h), with free access to water and chow (Nuvital, Porto Alegre, Brazil). All experimental procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol no. 13-0298) and conformed to the Guide for the Care and Use of Laboratory Animals (8th ed., 2011). Animal maintenance followed the Brazilian Law 11794 (specifying procedures for the use of animals in scientific research). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines [14].

2.2. Experimental design

The animals were acclimated to the study environment for 2 weeks before experimental initiation. It is important to emphasize that the application of Ac and EA in awake animals is complex and generates discomfort which may alter treatment-induced analgesia. Furthermore, anesthesia with isoflurane was used during treatments, and its use may have generated a bias in the study, considering the possible interference of the drug in behavioral and

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