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BRIEF REPORT

Retrosplenial Cortex is Involved in Analgesia Induced by 2- but not 100-Hz Electroacupuncture in the Rat Tail-Flick Test

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Abstract
This study examined whether or not the antinociceptive effect of 2- or 100-Hz electroacupuncture (EA) depends on the integrity of the retrosplenial cortex (RSC). Rats were taken for determination of tail-flick latency before and after injection of saline or 2% lidocaine (0.25 μl) into the retrosplenial cortex (RSC) bilaterally. Five minutes later, they were submitted to a 20-minute period of 2 Hz, 100 Hz, or sham EA at the Zusanli and Sanyinjiao acupoints bilaterally, and tail-flick latency was measured within 30 seconds after the end of stimulation and at 5-minute intervals for up to 30 minutes. EA at a frequency of either 2 or 100 Hz induced a strong and long-lasting inhibition of the tail-flick reflex in rats treated with saline (0.25 μl) injected into the RSC. The analgesia produced by 2-Hz EA lasted for a shorter time in lidocaine-treated rats. By contrast, RSC impairment did not change the analgesic effect of 100 Hz EA. The integrity of the RSC is implicated in the duration of analgesia induced by low-frequency EA but is not essential for the analgesic effects evoked by high-frequency EA.

1. Introduction

The nucleus raphe magnus, nucleus gigantocellularis-pars α, periaqueductal grey, anterior pretectal nucleus (APtN), and locus coeruleus are brain structures implicated in pathways that descend through the dorsolateral funiculus (DLF) to modulate nociceptive inputs in the spinal dorsal horn [1]. This system may also play a role in processing electroacupuncture (EA)-induced analgesia. Stimulation of these nuclei potentiates EA-induced analgesia, whereas lesions or neural block attenuates it [2]. Moreover, DLF lesion inhibits EA-induced analgesia.

Involvement of cortical structures in EA-induced analgesia has also been reported in the literature, and some of the cortical structures are also known to be involved in descending control of pain [3]. A neuroimaging study has shown that the retrosplenial cortex (RSC) is among the most consistently activated regions during noxious stimulation in rats [4]. The stimulation of the RSC induces antinociception in the rat tail-flick test, the effect depending, at least in

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part, on the integrity of the APtN, a nucleus that receives projections from the RSC, is tonically activated by persistent nociceptive inputs, and it is itself implicated in antinociception [5].

The mechanisms activated in the brain by EA differ according to the frequency of stimulation [2]. Therefore, this study examined whether or not the antinociceptive effect of 2- or 100-Hz EA in the rat tail-flick test depends on the integrity of the RSC.

2. Materials and methods

2.1. Animals

The experiments were conducted using male Wistar rats (140—160 g) from the main animal house of the University of São Paulo (Campus of Ribeirão Preto) and were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Number 103/2008).

2.2. Tail-flick test

Each animal was placed in a ventilated tube with the ventral surface of the tail (between 4 and 6 cm from the tip) laid across a wire coil maintained at room temperature ($23 \pm 2^{\circ}C$). The coil temperature was then raised by the passage of electric current, and the latency for the tail withdrawal reflex was measured. Each trial was terminated after 6 seconds to minimize the possibility of skin damage. Tail-flick latency (TFL) was measured at 5-minute intervals until a stable baseline was obtained in three consecutive trials.

2.3. Selection of animals

Each animal used in these experiments was preliminarily submitted to the tail-flick test before and after a 10-minute period of 2 Hz-EA applied to the Zusanli (ST36) and Sanyinjiao (SP6) acupoints. The animal was considered a "responder" when TFL measured during EA was at least 5.5 seconds. Further experiments were then performed 1 week later using only responder rats.

2.4. Implantation of cannula

Each animal was anesthetized with tribromoethanol (250 mg/ kg, i.p.) and a 12-mm length of a 23-gauge stainless-steel guide cannula was implanted into the skull until its tip lay 1 mm above the RSC using the following coordinates (in mm): AP = 3.0; L = 1.8; H = - 1.9. The implant was bilateral, and the cannulae were then fixed to the skull with two screws and dental cement. After receiving penicillin (50 mg/kg, i.m.), the animal was allowed to recover for at least 1 week before the experiments.

2.5. Electroacupuncture

Electroacupuncture was performed in rats lightly anesthetized with isoflurane (in oxygen flow through a loose-fitting, cone-shaped mask; 2% for induction and 0.5% for maintenance). Stainless steel acupuncture needles (0.3 mm in diameter and 30 mm in length) were inserted at a depth of 5 mm into each hind leg at the acupoints Zusanli (ST36, 5 mm lateral to the anterior tubercle of the tibia) and Sanyinjiao (SP6, 3 mm proximal to the medial malleolus, at the posterior border of the tibia). The stimuli were generated by a constant-current programmed pulse generator (EL608, NKL, Brusque, SC, Brazil) and applied for 20 minutes to both hind legs simultaneously. The electric stimuli were set as square waves with a width of 0.5 ms and frequency of 2 or 100 Hz. The current intensity was increased in a stepwise fashion until a muscle twitch was observed (around 150 μA). Similar procedures were performed on animals allocated to the sham EA groups, but no electrical current was applied to them.

2.6. Intracerebral injection

The drug or vehicle was microinjected intracerebrally using a glass needle (70—90 μm, o.d.) protected by a system of telescoping steel tubes. The assembly was inserted into the guide cannula, and the needle was advanced to protrude 1.0 mm beyond the guide cannula tip. The volume of the microinjection was 0.25 μl, which was delivered at a constant rate over a period of 3 minutes. Saline or 2% lidocaine was injected into the nucleus 5 minutes before the EA period.

2.7. Histology

At the end of the experiments, each animal was deeply anesthetized with intraperitoneal sodium thiopental and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate-buffered saline. Fast green (0.5 μl) was injected through the guide cannula. The brain was removed, and the dye spot was localized from 50-μm serial coronal sections stained with neutral red.

2.8. Statistical analysis

The tail-flick latencies are reported as means ± standard deviation. Comparisons between control and test groups were made by multivariate analysis of variance with repeated measures to compare the groups over all times. In the case of a significant treatment × time interaction, a one-way analysis of variance followed by the Bonferroni posthoc test was performed for each time.

3. Results

Thirty-six "responder" rats were used in this study. They all had histologically verified injector placements within the RSC. The latencies before and after exposure to isoflurane in each experimental group were not statistically different. Sham EA did not influence the TFL of rats treated with saline (control) or lidocaine injected into the RSC. The results from these animals served as control values relative to the effects of 2 or 100 Hz EA. A strong and long-lasting inhibition of the tail-flick reflex was induced by either