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Analgesia Induced by 2- or 100-Hz Electroacupuncture in the Rat Tail-Flick Test Depends on the Activation of Different Descending Pain Inhibitory Mechanisms

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Abstract: **We evaluated the effectiveness of intrathecal antagonists of α1- (WB4101) and α2- (idazoxan) adrenoceptors and serotonergic (methysergide), opioid (naloxone), muscarinic (atropine), $GABA_A$ (bicuculline) and $GABA_B$ (phaclofen) receptors in blocking 2- or 100-Hz electroacupuncture (EA)-induced analgesia (EAIA) in the rat tail-flick test. EA was applied bilaterally to the Zusanli and Sanyinjiao acupoints in lightly anesthetized rats. EA increased tail-flick latency, where the effect of 2-Hz EA lasted longer than that produced by 100-Hz EA. The 2-Hz EAIA was inhibited by naloxone or atropine, was less intense and shorter after WB4101 or idazoxan, and was shorter after methysergide, bicuculline, or phaclofen. The 100-Hz EAIA was less intense and shorter after naloxone and atropine, less intense and longer after phaclofen, shorter after methysergide or bicuculline, and remained unchanged after WB4101 or idazoxan. We postulate that the intensity of the effect of 2-Hz EA depends on noradrenergic descending mechanisms and involves spinal opioid and muscarinic mechanisms, whereas the duration of the effect depends on both noradrenergic and serotonergic descending mechanisms, and involves spinal GABAergic modulation. In contrast, the intensity of 100-Hz EAIA involves spinal muscarinic, opioid, and $GABA_B$ mechanisms, while the duration of the effects depends on spinal serotonergic, muscarinic, opioid, and $GABA_A$ mechanisms.**
Perspective: ***The results of this study indicate that 2- and 100-Hz EA induce analgesia in the rat tail-flick test activating different descending mechanisms at the spinal cord level that control the intensity and duration of the effect. The adequate pharmacological manipulation of such mechanisms may improve EA effectiveness for pain management.***

Key words: *Electroacupuncture, analgesia, descending pain control, spinal cord, tail-flick test.*

The spinal modulation of nociceptive inputs is mediated by serotonergic and noradrenergic fibers from several nuclei such as the nucleus raphe magnus (NRM), nucleus gigantocellularis pars α (Giα), nucleus reticularis paragigantocelullaris (NRPG), and locus coeruleus (LC) and subceruleus, which descend to the spinal cord passing through the dorsolateral funiculus (DLF).[21] Intrinsic spinal cholinergic, opioid and GABAergic neurons are also involved in the spinal modulation of nociceptive inputs.[61] Serotonin (5-HT) released from NRM fibers activate spinal enkephalinergic and GABAergic neurons,[33,37] whereas spinal cholinergic[12] and GABAergic[6,14] neurons are activated by norepinephrine (NE) released from noradrenergic fibers.

Pain inhibitory pathways are also activated by electroacupuncture (EA).[29,53] Stimulation of NRM, PAG or LC potentiates EA analgesia, whereas lesion or neural block of any of them attenuates it.[64] Moreover, DLF lesion inhibits EA analgesia in models of inflammatory,[29,32] visceral-somatic,[47] and phasic[48] pain.

The mechanisms activated in the brain by EA may differ according to the frequency of stimulation: lesion of the arcuate or supraoptic nuclei reduces low- but not high-frequency EA-induced analgesia, whereas lesion of the parabrachial nucleus produces the opposite effects;[18] low-frequency EA increases the spinal release of met-enkephalin, endomorphin and beta-endorphins, whereas high-frequency EA increases the spinal release of dynorphin.[33]

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The present study comparatively evaluated the effectiveness of intrathecal antagonists of NE, 5-HT, opioids, Ach, and GABA in blocking 2- or 100-Hz EA-induced analgesia in the rat tail-flick test. It is shown that 2- and 100-Hz EA produces antinociception that utilizes different descending mechanisms also at the spinal level. Portions of this study were presented at the 6th Congress of the European Federation of IASP Chapters.[49,50]

Methods

Animals

The experiments were conducted using male Wistar rats (140–160 g) from the main animal house of the University of São Paulo (USP; Campus of Ribeirão Preto). Animals were housed 2 to a cage under controlled temperature (24 ± 2°C) and on a 12-hour light-dark cycle, with the dark cycle beginning at 0700 hours; the animals had free access to food and water. The experiments were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Number 103/2008). The guidelines of the Committee for Research and Ethical Issues of IASP[65] were followed throughout the experiments. Each rat was used on only 1 occasion.

Tail-Flick Test

Each animal was placed in a ventilated tube with the tail laid across a wire coil maintained at room temperature (23 ± 2°C). The coil temperature was then raised by the passage of electric current and the latency for the tail-withdrawal reflex was measured. Heat was applied to a portion of the ventral surface of the tail between 4 and 6 cm from the tip. Each trial was terminated after 6 seconds to minimize the possibility of skin damage. Tail-flick latency was measured at 5-minute intervals until a stable baseline was obtained in 3 consecutive trials. Only rats showing stable baseline latency after up to 6 trials were used in each experiment.

Selection of Animals

Each animal used in these experiments was preliminarily submitted to the tail-flick test before and after a 5- and 10-minute period of EA applied to the Zusanli (ST36) and Sanyinjiao (SP6) acupoints at a frequency of 2 Hz as described elsewhere.[48] The animal was considered a responder[52] when tail-flick latency measured during EA was at least 75% above baseline (usually ≥5.5 seconds). The remaining rats were considered nonresponders to EA. Further experiments were then performed 1 week later using only responder rats.

Intrathecal Injection

The injections were performed in rats anesthetized with isoflurane (described elsewhere).[57] A 1-inch, 25-G needle was transcutaneously introduced at the L5-L6 level into the subarachnoid space.[35] A sudden lateral movement of the tail was taken as indicative that the needle entered the subarachnoidal space. A constant 5-μl volume was injected and the syringe was then held in position for a few seconds and gradually removed to avoid any outflow of the drug. All solutions contained 1% fast green dye to confirm the correct position of the catheter. Each rat received only 1 i.t. injection.

Electroacupuncture

The procedures were performed in rats lightly anesthetized with isoflurane (in oxygen flow through a loose-fitting, cone-shaped mask; 2% for induction, and .5% for maintenance) to minimize the stress induced by animal restraint.[55] Stainless steel acupuncture needles (size: .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 (NKL, Brusque, SC, Brazil) and applied for 20 minutes to both hind legs simultaneously. The electric stimuli were set as square waves, .5-ms width, and frequency of 2 or 100 Hz. The current intensity was increased in a stepwise fashion until a muscle twitch was observed (around 1500 μA) as proposed elsewhere.[59] Animals allocated into the sham EA groups were placed in the same apparatus and had needle insertion in the same acupoints, but no electrical current was applied to them, as proposed elsewhere.[22]

Experimental Protocol

Rats selected as responders to EA were taken for determination of baseline tail-flick latency (TFL). Each animal was then anesthetized with isoflurane and TFL measured 5 minutes after the beginning of the maintenance period. Only rats showing similar pre- and post-anesthetic TFL were considered for further analysis. Vehicle (saline or DMSO) or antagonist was then injected intrathecally, and 5 minutes later, TFL was measured once again. Rats were then submitted to a 20-minute period of EA. TFL was measured within 30 seconds after the period of stimulation and at 5-minute intervals for up to 30 minutes. No attempt was made to measure tail-flick latency during the EA period.

Drugs

The following drugs were used. WB 4101 hydrochloride, idazoxan hydrochloride, methysergide maleate, naloxone hydrochloride, bicuculline methiodide, atropine sulfate, and phaclofen were purchased from RBI/Sigma (Natick, MA, USA). Phaclofen was dissolved in 5% dimethyl sulfoxide (DMSO). The other drugs were dissolved in saline.

Histology

At the end of the experiment, each animal was deeply anesthetized with intraperitoneal sodium thiopental and perfused through the heart with 4% paraformaldehyde in .1 M phosphate buffered saline. Incorrect injections were detected by the presence of fast green dye in the paravertebral musculature or in the dorsal