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Analgesia induced by 2- or 100-Hz electroacupuncture in the rat tail-flick test depends on the anterior pretectal nucleus

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ABSTRACT

Aims: The anterior pretectal nucleus (APtN) and electroacupuncture (EA) activate descending mechanisms to modulate nociceptive inputs in the spinal dorsal horn. This study examines qualitatively whether mechanisms in the APtN participate in the EA-induced analgesia in rats.
Main methods: The tail-flick test was utilized to examine the changes produced by non-selective antagonists of serotonergic (methysergide, 37 pg), muscarinic (atropine, 10 ng) and opioid (naloxone, 10 ng) receptors; selective antagonists against μ (CTOP, 6.4 μg), δ (ICI174,864, 6.9 μg) or κ (nor-BNI, 7.3 μg); $5HT_1$ (methiothepin, 0.47 μg), $5HT_2$ (ketanserin, 5.4 μg), or $5HT_3$ (MDL 72222, 15.7 μg); and $GABA_A$ (bicuculline, 150 ng) receptors injected into the dorsal (d) or ventral (v) APtN on the antinociception induced by a 20-min EA applied at 2- or 100-Hz frequency to the Zusanli and Sanyinjiao acupoints.
Key findings: The 2-Hz EA-induced analgesia was blocked by naloxone, CTOP or atropine, was less intense after bicuculline, was shorter after methysergide or methiothepin in dAPtN, and was less intense after methysergide, methiothepin and bicuculline in vAPtN. The 100-Hz EA-induced analgesia was less intense after methysergide, methiothepin and CTOP in vAPtN, and remained unchanged after injection of the antagonists into the dAPtN.
Significance: The 2-Hz EA-induced analgesia utilizes cholinergic muscarinic, μ-opioid, $GABA_A$ and 5-HT_1 mechanisms in the dAPtN and μ-opioid and 5-HT_1 mechanisms in the vAPtN, while 100-Hz EA-induced analgesia utilizes μ-opioid and 5-HT_1 mechanisms in the vAPtN but does not utilize them in the dAPtN.

Introduction

The anterior pretectal nucleus (APtN) is a midbrain structure involved in nociception and antinociception, and in pathways that descend through the dorsolateral funiculus (DLF) to modulate nociceptive inputs in the spinal dorsal horn (see Rees and Roberts, 1993). A role for APtN in nociception is supported by the demonstration that high intensity peripheral noxious stimulation excites APtN neurons (Rees et al., 1995) and increases the number of c-Fos positive cells in the APtN (Villarreal et al., 2003) and the uptake of [^{14}C]-deoxyglucose by APtN cells (Porro et al., 1995; Neto et al., 1999). On the other hand, brief electrical stimulation of the APtN produces strong and long-lasting antinociceptive effects in the rat tail-flick (Prado and Roberts, 1985; Roberts and Rees, 1986), formalin (Wilson et al., 1991) and post-incision (Villarreal et al., 2003, 2004; Villarreal and Prado, 2007) tests.

The APtN is anatomically divided into rostral–dorsal and ventral–caudal parts (Foster et al., 1989; Cadusseau and Roger, 1991). Stimulation of the dorsal APtN is the most effective part of the nucleus in the tail flick test, whereas stimulation at the ventral APtN is the most effective against a persistent incisional pain (Villarreal et al., 2004). Muscarinic cholinergic, μ-opioid and 5-HT_{1B} serotonergic mechanisms within the APtN were shown to modulate nociceptive and antinociceptive inputs (Prado, 1989; Rees et al., 1992; Rosa and Prado, 1997). A hypothetical model postulates that noxious inputs drive cholinergic or serotoninergic terminals in the APtN to activate distinct descending pathways, the serotonergic pathway being under inhibitory GABAergic control, which in turn is negatively driven by opioid nerve terminals (Villarreal and Prado, 2007).

The APtN is involved also in the mechanism of electroacupuncture (EA)-induced analgesia (EAIA) (Zhu et al., 2004). Although not fully understood, EAIA involves the activation of descending modulation of pain mechanisms (Toda, 1982; Lee et al., 2007), since DLF lesion inhibits EAIA in several models of pain (Li et al., 2005; Shen et al., 1975; Lee et al., 2007; Silva et al., 2010). The mechanisms activated by EA differ according to the frequency of stimulation. Low-frequency EA (2–10 Hz) increases the spinal release of met-enkephalin, endomorphin and beta-endorphins, whereas high-frequency EA (50–100 Hz) increases the spinal release of dynorphin (Fei et al., 1987; Han, 2004).

The 2-Hz EAIA did not occur in rats with neural block of the whole or dorsal APtN, whereas the 100-Hz EAIA was reduced in rats with neural

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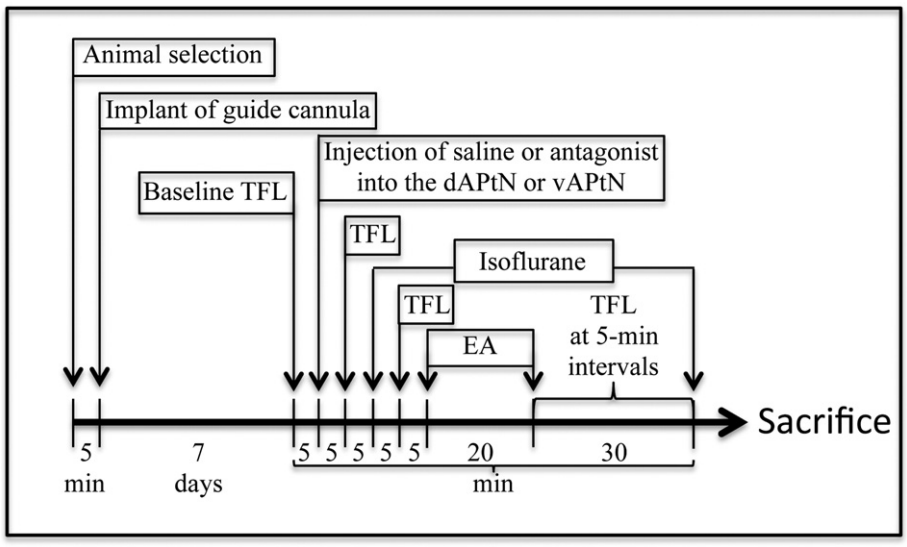

Fig. 1. Summary of the protocol used in the study. Abbreviations: EA = electroacupuncture; TFL = tail-flick latency; dAPtN = dorsal anterior pretectal nucleus; and vAPtN = ventral anterior pretectal nucleus.

block of the whole APtN, but remained unchanged in rats with neural block of the dorsal APtN (Silva et al., 2010), thus leading to the notion that different mechanisms in the APtN may be implicated in EAIA. This study therefore utilizes the tail-flick test to examine qualitatively whether cholinergic muscarinic, opioid, serotonergic and GABA mechanisms in the dorsal and ventral APtN participate in the analgesia evoked by 2- and 100-Hz EA applied to the Zusanli and Sanyinjiao acupoints in rats.

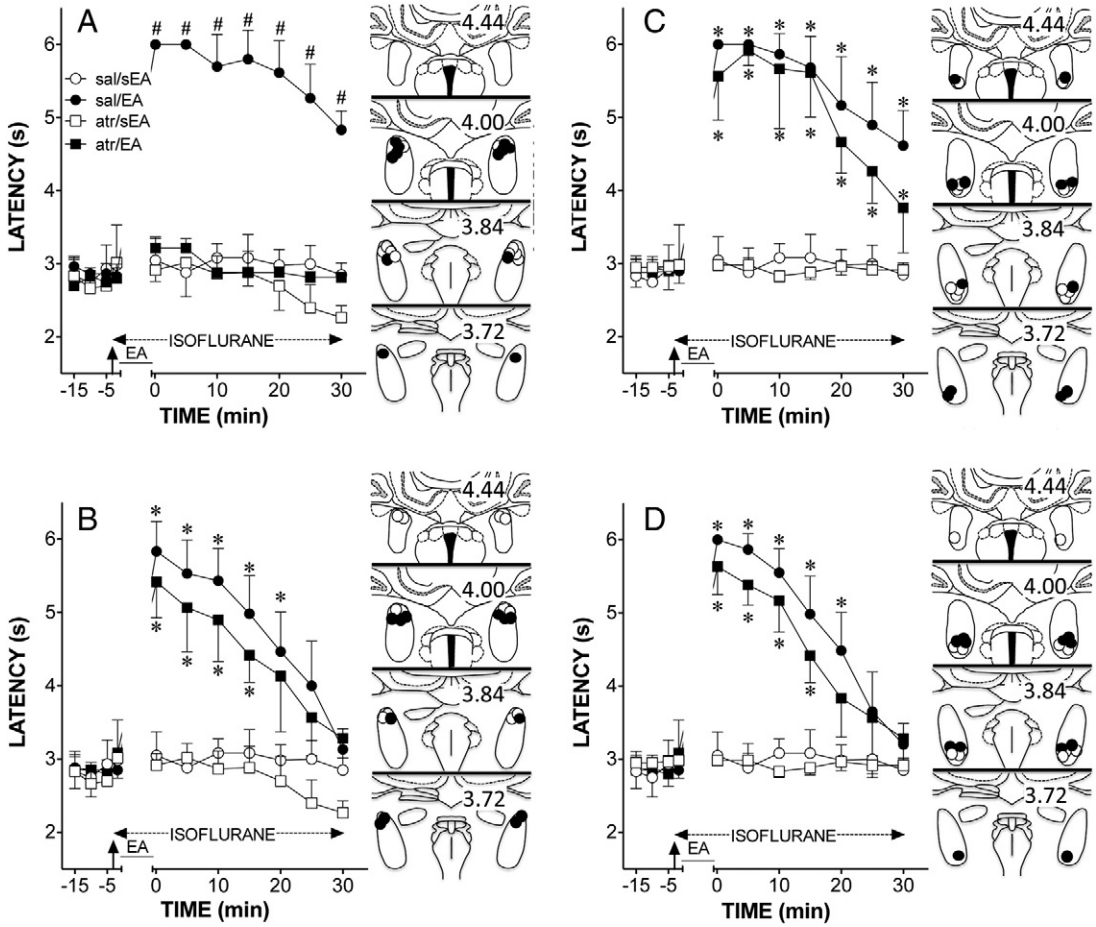

Fig. 2. The changes induced by atropine on the electroacupuncture (EA)-induced analgesia on the tail-flick latency of rats anesthetized with isoflurane. Saline (sal = 0.10 μl) or atropine (atr = 10 ng/0.10 μl) was injected into the dorsal (A and B) or ventral (C and D) anterior pretectal nucleus at the moment indicated by a vertical arrow. EA or sham EA (sEA) was applied during 20 min (solid horizontal line) at 2 Hz (A and C) or 100 Hz (B and D) frequency. The localization of injection sites of each graph is shown at the right on diagrams taken from Paxinos and Watson (2004). Points are means (±SD) of six rats per group. $P < 0.05$ compared to control (sal/sEA) (*) or any other group (#).