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Research article

Intrathecal nefopam-induced antinociception through activation of descending serotonergic projections involving spinal 5-HT7 but not 5-HT3 receptors

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HIGHLIGHTS

- Intrathecalnefopam has an antinoicieptive effect in formalin and paw incision test.
- Nefopam's antinociception involves spinal 5-HT7 receptor, but not 5-HT3 receptor.
- Spinal 5-HT plays a vital role in nefopam's antinociception in paw incision test.
- Role of spinal 5-HT in nefopam's antinociception in formalin test is indeterminate.

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ABSTRACT

We examined the involvement of spinal 5-HT(5-hydroxytryptamine) receptor 3(5-HT3R) and 7(5-HT7R) as well as the overall role of descending serotonergic projections in the analgesic effects of intrathecal(i.t.) nefopam for two rat models of formalin and paw incision test. I.t. nefopam produced an antinociceptive effect in a dose-dependent manner in both tests. Lesioning the spinal serotonergic projections using i.t. 5,7-dihydroxytryptamine(5,7-DHT) did not influence the intensity of allodynia in the paw incision test, but i.t. 5,7-DHT abolished the effect of nefopam. In the formain test, i.t. 5,7-DHT alone significantly diminished the flinches, but the effect of nefopam was not affected by i.t. 5,7-DHT. Antagonism study showed that i.t. 5-HT7R antagonist, SB269970 significantly blocked the antinociceptive effect of nefopam in both tests, but i.t. 5-HT3R antagonist, ondansetron has no influence on the effect of nefopam. The present study demonstrates that descending spinal serotonergic projections play a vital role in antinociceptive effect of i.t. nefopam in the paw incision test, but indeterminate in the formalin test. In both tests, the antinociceptive effect of i.t. nefopam involves the spinal 5-HT7R, but not 5-HT3R.

1. Introduction

Nefopam is a centrally acting, non-opioid and non-steroidal analgesic drug. It has been widely used to control postoperative pain [8]. Inhibition of reuptake of monoamines including serotonin has been suggested as the main mechanism of the nefopam-induced antinociception [9,21], but conflicting results regarding the contribution of 5-hydroxytryptamine (5-HT) in the spinal cord to the action of nefopam have been presented. Lesioning in descending serotonergic projections reduced the antinociceptive effect of nefopam in acute phase of formalin test [13], but did not influence its effect in neuropathic pain of rat [4]. In addition, the involvement of specific 5-HT receptor subtypes in the analgesic effect of nefopam is still unclear.

The use of a selective 5-HT3R antagonist in combination with nefopam could attenuate nausea and vomiting, one of the common adverse effects of nefopam during postoperative pain management [23]. Some studies investigated the effect of ondansetron on analgesic effects of several drugs with different results according to drug and type of pain [10,11,20,25]. Blockade of 5-HT3R was shown not to alter the antinociceptive effect of nefopam in acute pain of rat

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formalin test [11], but no such reports regarding facilitated state of the formalin test or incision pain have been provided.

5-HT7R, the most recently identified 5-HT receptor, has been shown to play a significant role in the analgesic effect of several drugs including tramadol [6,29], which has been known to inhibit the reuptake of serotonin [22]. Furthermore, the blockade of 5-HT7R reduced the antiallodynic effect of intrathecal(i.t.) nefopam in spinal nerve ligation model [4]. However, the role of 5-HT7R in the effect of nefopam is still inconclusive because another study using the same model also demonstrated that the 5-HT7R antagonist reduced the tactile allodynia in the same neuropathic pain model [1].

This study investigated the role of the descending serotonergic projections and spinal 5-HT3Rand 5-HT7R in the antinociceptive effect of i.t. nefopam on formalin-evoked and incision pain in rats.

2. Material and methods

2.1. Animals

The present study was performed according to International Association for the Study of Pain guidelines for the Use of Animals in Research. Male Sprague–Dawley rats weighing 225–250 g were housed in a room with constant temperature of 22–23 °C and an alternating 12 h light/dark cycle with unrestricted access to food and water. Implantation of i.t. catheter was performed for drug administration under sevoflurane anesthesia. Rats showing neurological deficits were sacrificed immediately with an overdose of sevoflurane. All experiments were carried out by an observer blind to the treatments.

2.2. Nociceptive tests and behavioral testing

In the formalin test, the plantar surface of the hind paw was subcutaneously injected with 50 μL of 5% formalin solutions [30]. Characteristic biphasic flinching responses of phase 1 (acute pain) and phase 2 (facilitated pain) were recorded every 5 min.

The surgery for the paw incision test includes an incision through the plantar skin and fascia and plantaris muscle as previously described [3]. Von Frey test were used for determining the 50% probability paw withdrawal threshold (PWT) by the up and down method, in which a positive response was defined as brisk withdrawal or paw flinching during or immediately after application of a filament.

2.3. Drugs and lesioning the spinal serotonergic projections

Nefopam (Pharmbio, Korea) and ondansetron (5-HT3R antagonist; Tocris, UK) were dissolved in saline, and SB-269970 (5-HT7R antagonist; Tocris, UK) in 10% dimethyl sulfoxide and diluted with saline. All drugs were delivered with a gear-operated syringe pump in a 10 μL volume of solution followed by an additional 10 μL normal saline to flush the catheter.

Lesioning of spinal serotonergic projections was performed 72 h before intraplantar injection of carrageenan using 5,7-DHT (Sigma–Aldrich, USA), which ablates serotonergic nerve fibers and depletes 5-HT in the spinal cord [19]. Desipramine (30 mg/kg, Sigma–Aldrich, USA) was injected intraperitoneally 45 min before the i.t. injection of 5,7-DHT (60 μg/20 μL) to prevent non-specific uptake of 5,7-DHT by noradrenergic nerve fibers. 5,7-DHT was dissolved in saline containing 0.1% ascorbic acid, and desipramine in saline. Depletion of 5-HT in the dorsal half of lumbar enlargement was confirmed using a high-performance liquid chromatography system (Shimadzu Inc. Japan) coupled to a triple quadrupole mass spectrometer (API 4000 QTRAP, Applied Biosystems, USA).

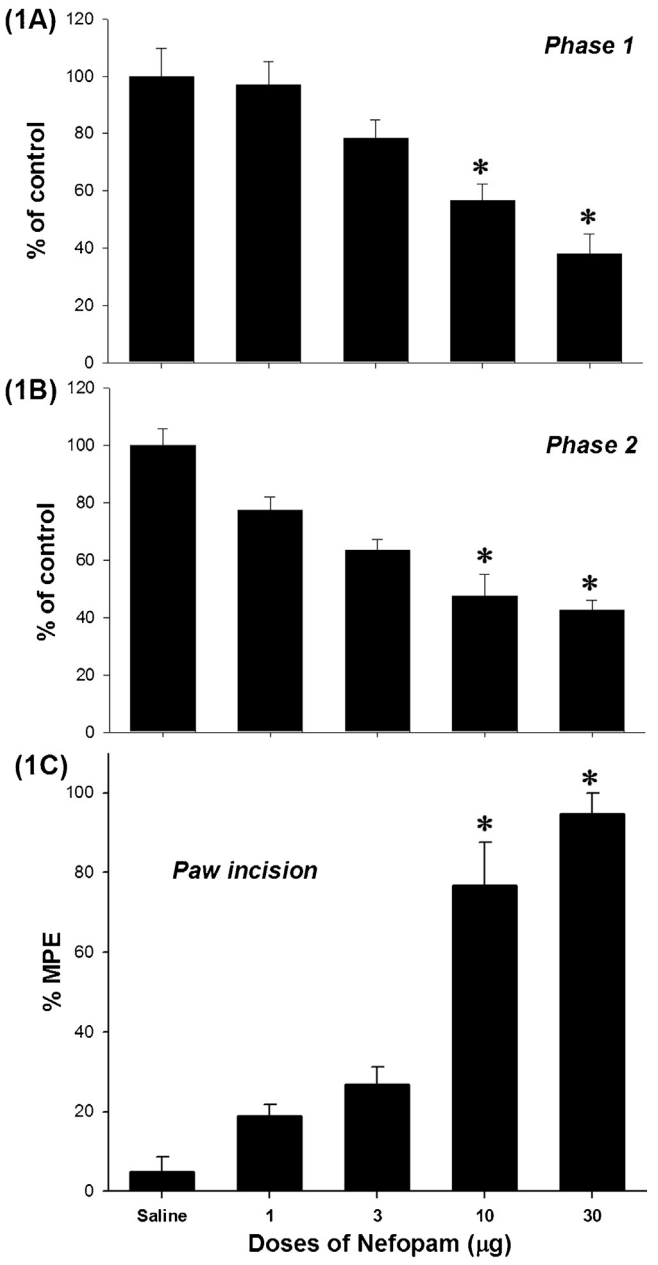

Fig. 1. Intratehcal nefopam significantly attenuated the pain behavior in phase 1 ((1A); $F = 12.282$, $P < 0.05$) and phase 2 ((1B); $F = 18.989$, $P < 0.05$) of formalin ($n = 7$ each dose) and paw incision test ((1C); $F = 44.234$, $P < 0.05$; $n = 6$ each dose). Flinches or PWT were converted to % of control (percentage of control) or %MPE (percentages of maximal possible effect), respectively. PWT; paw withdrawal threshold. *$P < 0.05$ vs. saline.

2.4. Experiment protocol

For testing the antinociceptive effect of nefopam, it was given intrathecally 10 min before intraplantar formalin injection, or following testing post-injury baseline PWT 120 min after paw incision. In the second experiment, we evaluated the antinociceptive effects of i.t. nefopam (30 μg) in 5,7-DHT treated rat. Finally, ondansetron (30 μg) or SB269970 (10 μg) were administered intrathecally 10 min before i.t. nefopam (30 μg) to determine the involvement of the 5-HT3R and 5-HT7R in the effects of i.t. nefopam. The doses of antagonists were determined from pilot experiments of our laboratory, in which the doses selected in the current study have no effect on normal and pain behavior.