Spinal Noradrenergic Modulation and the Role of the Alpha-2 Receptor in the Antinociceptive Effect of Intrathecal Nefopam in the Formalin Test

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Background:
Nefopam has shown an analgesic effect on acute pain including postoperative pain. The reuptake of monoamines including serotonin and noradrenaline has been proposed as the mechanism of the analgesic action of nefopam, but it remains unclear. Although alpha-adrenergic agents are being widely used in the perioperative period, the role of noradrenergic modulation in the analgesic effect of nefopam has not been fully addressed.

Methods:
Changes in the antinociceptive effect of intrathecal (i.t.) nefopam against formalin-elicited flinching responses were explored in Sprague-Dawley rats pretreated with i.t. 6-hydroxydopamine (6-OHDA), which depletes spinal noradrenaline. In addition, antagonism to the effect of nefopam by prazosin and yohimbine was evaluated to further elucidate the antinociceptive mechanism of i.t. nefopam.

Results:
Pretreatment with i.t. 6-OHDA alone did not alter the flinching responses in either phase of the formalin test, while it attenuated the antinociceptive effect of i.t. nefopam significantly during phase 1, but not phase 2. The antagonist of the alpha-2 receptor, but not the alpha-1 receptor, reduced partially, but significantly, the antinociceptive effect of i.t. nefopam during phase 1, but not during phase 2.

Conclusions:
This study demonstrates that spinal noradrenergic modulation plays an important role in the antinociceptive effect of i.t. nefopam against formalin-elicited acute initial pain, but not facilitated pain, and this action involves the spinal alpha-2 but not the alpha-1 receptor. (Korean J Pain 2014; 27: 23-29)

Key Words:
alpha-2 receptor, formalin, nefopam, noradrenergic system, spinal cord.
INTRODUCTION

Nefopam, a centrally acting non-opioid agent, has been widely used in European countries, and it could have useful applications in clinical practice due to its analgesic mechanism, which differs from that of other analgesic agents. It has been proposed in anecdotal literature to act through inhibition of monoamine reuptake, but the underlying mechanism of action remains inconclusive [1-5].

A recent study, which investigated the role of specific subtypes of the monoamine system in the antinociception of systemic nefopam for phase 1 of the formalin test, demonstrated a significant involvement of the dopamine D2 receptor, but not of alpha-1 or alpha-2 adrenergic receptors, in the formalin test [6]. However, there have been no such reports regarding phase 2 of the formalin test. Most of the previous studies did not look into nefopam’s mechanism of action at the spinal level even though the spinal cord is also an important site of action of monoamines which mediate descending pain modulation [7,8]. Furthermore, previous reports demonstrated the analgesic effect of spinal nefopam [9-11], but the overall role of the spinal noradrenergic system in producing nefopam’s analgesic effect remains unclear.

Several reports have demonstrated the benefits of using nefopam in clinical settings, which include synergistic analgesic interaction with opioids, a preventive effect on postoperative shivering, and a lack of analgesic antagonism by the antiemetic 5-HT3 receptor (5-hydroxytryptamine receptor subtype 3) antagonist, ondansetron [9,12-15]. Although alpha-adrenergic agents are being widely employed in the perioperative period, the role of noradrenergic modulation in the analgesic effect of nefopam has not been fully addressed. Furthermore, a recent study also demonstrated the important role of the alpha-2 receptor in the anti-shivering effect of nefopam, suggesting the possibility that the alpha-2 receptor is involved in nefopam’s mechanism of analgesia [16].

This study was performed to evaluate the role of the spinal noradrenergic system and alpha-1 and alpha-2 receptors in the antinociceptive effect of nefopam at the spinal level.

MATERIALS AND METHODS

1. Animals and intrathecal catheter implantation

Male Sprague-Dawley rats weighing 225–250 g were housed in a room with a constant temperature of 22–23°C and an alternating 12-hour light/dark cycle. Free access to water and food was allowed. All experiments were performed according to the International Association for the Study of Pain Guidelines for the Use of Animals in Research.

A polyethylene-5 (PE-5) catheter was implanted into the intrathecal (i.t.) space to allow administration of the experimental drugs as described in the previous study [9]. Under anesthesia with inhalation of sevoflurane, the dorsal part of the neck was dissected to reveal the atlantooccipital membrane and a PE-5 catheter was introduced through the membrane. The catheter was then advanced caudally 8.5 cm to the level of the lumbar enlargement. The other end of the catheter was exteriorized to the top of the head and plugged with wire for drug administration. Any rats showing neurological deficits after the surgery were sacrificed immediately with an anesthetic overdose. Each rat was given a subcutaneous injection of 5 ml saline before the end of the surgery, and the animals were housed in individual cages following the procedure.

2. Nociceptive test and behavioral study

The animals were restrained in a cylinder and were then injected subcutaneously with 50 μl of 5% formalin into the center of the hindpaw using a 30 gauge needle. Following the intraplantar injection of formalin, the animals displayed a typical flinching response, which represents initial acute nociception (phase 1, acute pain) by direct stimulation of the peripheral nociceptors, and a following facilitated state of spinal dorsal horn neurons as well as peripheral sensitization (phase 2, facilitated pain) [17]. After an animal was injected, an observer counted the number of flinching responses at 1 min and 5 min (phase 1, 0–9 min), and at 5-min intervals between 10–60 min (phase 2, 10–60 min). Each counting of flinching responses was of 1-min duration. The observer who carried out the behavioral testing was blinded to the agents used for i.t. treatment.

3. Drugs

This experiment used the following drugs: prazosin (alpha-1 receptor antagonist), yohimbine (alpha-2 receptor antagonist), and nefopam hydrochloride (Acupan®). The vehicle for the drugs was saline. All of the drugs were administered using a hand-driven gear-operated syringe pump through an i.t. catheter in a volume of 10 μl, followed