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SPINAL ANTINOCICEPTIVE ACTION OF LOPERAMIDE IS MEDIATED BY OPIOID RECEPTORS IN THE FORMALIN TEST IN RATS

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Abstract

Opioids like morphine produce antinociception after intrathecal administration. Being hydrophilic in nature, morphine also spreads rostrally and produces respiratory depression. Loperamide has been reported to produce antinociception after both intracisternal and intrathecal administration. It is also hydrophobic, which could restrict its diffusion in the spinal canal. However, the mechanism of its antinociceptive action after intrathecal administration is not definitely known. In the present study, the antinociceptive effect of loperamide was evaluated by the formalin test. It significantly inhibited Phase II flinching behavior. This antinociceptive effect was reversed by pre-administration of naloxone indicating that it was predominantly due to activation of opioid receptors.

Keywords

Intrathecal; Morphine; Mu-opioid receptor; Naloxone; Respiratory depression; Voltage-sensitive calcium channel

Introduction

Opioids like morphine produce antinociception after intrathecal administration in experimental animals and in humans [18-19]. This antinociceptive effect is the basis of spinal analgesia for treating severe chronic pain as in cancer patients [6]. The analgesic effect is due to the direct interaction of the opioid drugs with muopioid receptors, expressed by primary afferent terminals and neurons in the superficial part of the dorsal horn (Laminae I-II) in the spinal cord [3]. However, intrathecally administered drugs also migrate cranially and caudally within the spinal canal and produce side effects like respiratory depression and urinary retention. This applies particularly to drugs like morphine, which are hydrophilic in nature [1]. On the contrary, hydrophobic drugs like fentanyl (log P = 4.25) demonstrate less migration and lesser incidence of respiratory depression, though they are cleared quickly into the plasma [2]. Loperamide is also a hydrophobic drug with a high log P (3.9), which results in its sequestration in proximal lipid membranes. So significant is this sequestration that it does not cross the bloodbrain barrier. However, it has been reported to produce significant antihyperalgesic effect after local peripheral administration in local inflammation models (knee joint and paw inflammation) and the formalin test [4] though it does not attenuate allodynia after peripheral nerve injury [15]. Similarly, intraplantar administration of loperamide could abolish thermal hyperalgesia but not allodynia in mice osteosarcoma model induced by inoculation of NCTC 2472 cells into the

tibia [11]. The analgesic effect was observed to be mediated by opioid receptors [4,7,11,15]. In addition to its peripheral action, neuraxial (intracisternal/intrathecal) administration of loperamide also leads to antinociception [8,14]. The present study also investigates the antinociceptive effect after intrathecal administration of loperamide though with certain differences from the earlier report [8]. These are regarding the animal model used for the study (6-8 weeks old mice in the previous study vs 8-9 weeks old rats in the present study), the mode of administration of the drug (intrathecal percutaneous injections vs administration by intrathecal catheter) and the specific antinociceptive behavior (licking time vs flinching in the formalin test) observed during the study. The formalin test is probably the most common chemical assay for nociception in laboratory animals [9]. In this, a dilute solution of formaldehyde is injected into the rat hind paw followed by observation of specific pain-related behavior like flinching over a period of 1 hour [5,17]. Furthermore, while some have shown naloxone reversibility [14], others have reported that the analgesic action of centrally administered loperamide is resistant to reversal by naloxone [8]. Naloxone is a competitive antagonist of the opioid receptors. Instead, the analgesic effect was noted to be due to the blocking action of loperamide on multiple voltage-sensitive calcium channels. The aim of the present study was to investigate the antinociceptive effect of loperamide after acute intrathecal administration through an indwelling catheter in the rat formalin test. Besides, naloxone reversibility of its antinociceptive action was also studied.

Materials and methods

The experimental protocol was approved by the Institutional Animal Care Committee of University of California, San Diego. Male Holzman Sprague-Dawley rats (weight: 275-350 gm; 8-9 weeks old), kept in alternating 12h each of light-dark cycle, were used for the present study. Food and water were available *ad libitum*. Under isoflurane anesthesia, these rats were implanted with intrathecal catheters (PE-5, 8.5 cm) through the cisternal membrane as described earlier [20]. The outer end was plugged with a metal wire. After recovery for 5 days, the rats displaying normal motor functions were entered into the study.

To assess formalin evoked flinching, an automated system for counting the flinching behavior was used [21]. A metal band was put around the right hind paw and 50 μ L of 2.5% formalin solution was injected subcutaneously in the dorsal surface. Flinches were counted in 1 min bins for 60 min. These were divided into Phases I (0-9 min) and II (10-60 min). Phase II was further divided into Phase IIA (10-39 min) and IIB (40-60 min).

Loperamide hydrochloride (Sigma-Aldrich, St. Louis, USA) was dissolved in a vehicle consisting of polyethylene glycol, normal saline and ethyl alcohol in a ratio of 2:2:1, which had been previously standardized [16]. In this study, the vehicle did not show an antinociceptive effect. Different doses of the drug (3 μ g, 10 μ g and 30 μ g) were administered in a volume of 10 μ L through the intrathecal catheter, which was flushed with physiological saline (0.9%). Intraplantar formalin injection was performed, 30 min after intrathecal administration of loperamide. This was done on the basis of preliminary experiments that showed peaking of the antinociceptive effect 30 min after administration. For control experiments, physiological saline was injected instead of loperamide. For naloxone reversibility, naloxone (3 mg/kg intraperitoneally) was administered 10 min before intrathecal loperamide administration. Naloxone reversibility was evaluated with the highest dose of loperamide (30 μ g).

The data was analyzed by Student's *t*-test using the program Prism (GraphPad software, San Diego, CA). Significance was set at $P < 0.05$.

Results

Formalin injection in saline treated rats produced a typical biphasic response. Increased number of flinches were noted between 0-5 min and again between 20-40 min (Fig 1a). Administration of both 3 and 10 µg of loperamide did not significantly change the flinching behavior. However, 30 µg loperamide significantly reduced the flinches between 30-35 and 40-45 min. Phase-wise analysis indicated significant reduction in Phase II (Fig 1b). Within Phase II, Phase IIB showed a greater inhibition than Phase IIA. Notably, naloxone reversed the antinociceptive effect of 30 µg of loperamide in Phase IIB (Fig 1c). Naloxone alone had no effect upon formalin flinching (data not shown).

Discussion

The results of the present study indicate that intrathecal loperamide (only at 30 µg but not at 3 or 10 µg) is only effective in Phase II of the formalin test. Phase II is associated with facilitated pain processing in the spinal cord [17]. Our results are in contrast to an earlier study, which had reported that intracisternal administration of loperamide inhibited both phases of the formalin test [14]. It was also observed by the earlier authors that lower doses of loperamide (5 and 10 µg for phase I and 10 and 20 µg for Phase II) were effective. The discrepancy may be related to the route of administration of the drug as intracisternal injection might have activated both spinal and supraspinal opioid receptors by diffusion of the drug. Supraspinal administration of opioids modifies the aversive-affective component of pain besides inhibiting pain transmission at the level of the brain stem, thalamus and cortex, which could be additional to its action on the spinal cord [10].

Naloxone (3 mg/kg) could significantly reverse the antinociceptive effect of loperamide in our study. It indicated that loperamide was predominantly acting through an opioid receptor. Similar finding has been earlier reported, in which, naloxone (1 mg/kg subcutaneously) could reverse the effect of intracisternal administration of loperamide [14]. However, in our previous study involving antinociceptive effect of intrathecal loperamide on the tail-flick test, partial reversibility was noted, which indicated that other mechanisms like blockade of voltage-sensitive calcium channels could be responsible as reported earlier by Hagiwara et al. (2003) [8,16]. Interestingly, in the study by Hagiwara et al. 2003 in mice, naloxone could not reverse the antinociceptive effect of loperamide and they speculated the role of a direct effect upon channel function. As the formalin test is related to tissue injury, it is considered to be a more valid model of clinical pain than those assays dependent on brief thermal stimulation [12]. Loperamide is peripherally restricted i.e., does not cross the blood brain barrier, which prevents its abuse [13]. This, along with its antinociceptive effect after intrathecal administration, indicates that it might be a useful drug for treating moderate to severe pain in cancer patients.

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Fig 1.

Analysis of flinching behavior of rats in the formalin test (a) Total number of flinches have been shown in 5 min bins. As compared to saline treated group (control), intrathecal administration of only 30 μ g of loperamide produced significant inhibition at 35 and 45 min as compared to the control group. (b) Analysis of flinching behavior according to different phases. Loperamide produced significant inhibition in Phase II as compared to control. Specifically, it inhibited flinches in Phase IIB. (c) Naloxone reversibility of flinching behavior. Naloxone significantly reversed flinching behavior in Phase II. Specifically, it inhibited flinches in Phase IIB. This is in concordance with the results shown in (b), where loperamide (30 μ g) produced inhibition in the same Phases. Values are shown as mean $\pm$ S.E.M.