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Antinociceptive effect of intrathecal loperamide: Role of mu-opioid receptor and calcium channels

Rakesh Kumar [a], K.H. Reeta [b], Subrata Basu Ray [a,*]

[a] Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India
[b] Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-110029, India

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ABSTRACT

Morphine is a gold standard analgesic commonly used to alleviate pain. However, its use is associated with unavoidable side effects including the risk for addiction. Peripherally administered loperamide lacks effect on the central nervous system as it is a substrate for the permeability glycoprotein (P-gp) efflux pump which blocks its entry into brain. However, when administered intrathecally, loperamide has been reported to produce analgesia. The present study investigates the mechanism of the central analgesic effect of loperamide. Adult male Sprague-Dawley rats were subjected to surgery for catheter placement. Following baseline testing, different groups of rats were administered fixed intrathecal doses (1 μg, 3 μg, 10 μg and 30 μg) of loperamide and morphine. Analgesia was compared employing Hargreaves paw withdrawal apparatus at 15 min, 30 min, 60 min, 90 min and 120 min. Additionally, CTOP, a specific mu-opioid receptor antagonist was co-administered with loperamide to examine the mu-opioid receptor mediated loperamide analgesia. Furthermore, nefiracetam, a calcium channel opener, was co-administered with loperamide or morphine to evaluate the involvement of Ca^{2+} channels in Loperamide showed an analgesic effect which was comparable to morphine. However, loperamide produced longer analgesia and the analgesic effect was significantly better at 42 h and 49 h compared to morphine. CTOP completely reversed loperamide analgesia. Though nefiracetam significantly reversed loperamide analgesia, it did not have any effect on morphine induced analgesia. Our findings suggest that loperamide administered intrathecally produces analgesia which is mediated through mu-opioid receptor and subsequent blockade of downstream calcium channels.

1. Introduction

Pain is a biological process arising from damage or disease in the body. Acute pain in particular warns individuals of possible danger and strengthens the protective mechanism to minimise injury. Morphine is the gold standard for pain treatment in clinical settings but its long-term use is associated with severe side-effects and tolerance (Osenbach and Harvey, 2001).

Loperamide, an opioidergic drug used for treating non-bacterial diarrhoea (Niemegeers et al., 1974) has been reported to produce significant antihyperalgesic effect after peripheral administration in local inflammation models (knee joint and paw inflammation) and in the formalin test (Dehaven-Hudkins et al., 1999). However, it did not attenuate allodynia after peripheral nerve injury (Shinoda et al., 2007). Moreover, intraplantar administration of loperamide could abolish thermal hyperalgesia but not allodynia in mice osteosarcoma model (Menéndez et al., 2007). Analgesia of peripherally administered loperamide has been shown to be reversed by opioid antagonists entailing that loperamide analgesia is mediated through opioid receptors (Dehaven-Hudkins et al., 1999; Shinoda et al., 2007; Guan et al., 2008). Loperamide does not cross blood brain barrier (BBB) (Schinkel et al., 1996). However, its intrathecal administration produced analgesia in the formalin (Shannon and Lutz, 2002; Hagiwara et al., 2003; Ray and Yaksh, 2008) and tail flick (Ray et al., 2005) tests.

In the present work, we investigated the analgesic effect of intrathecal loperamide and compared it with that of morphine. We used the Hargreaves paw withdrawal apparatus for the purpose, and the latency of paw withdrawal was recorded. Following intrathecal administration, drug can migrate in rostral and caudal direction to influence various centers in brain. Hargreaves apparatus has an advantage of testing the spinal and supraspinal action of a drug. The mechanism of loperamide analgesia was also determined using D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH(2) (CTOP), a specific mu-opioid receptor antagonist (Hawkins et al., 1989) and nefiracetam, an activator of voltage-gated calcium channels (VGCCs) (Yoshii and Watabe,

* Corresponding author. Tel.: +91 11 26593453; fax: +91 11 26588663., 26588641.
E-mail address: raysb48@gmail.com (S.B. Ray).

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1994). The total duration of analgesic action of single dose of morphine and loperamide (30 μg each) was tested at the successive time intervals until it returned to baseline.

2. Materials and methods

2.1. Animals and surgery

Adult male Sprague-Dawley rats (275–350 g) were obtained from the Institutional Central Animal Facility. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), All India Institute of Medical Sciences, New Delhi, India. Animals were kept under standard laboratory conditions in natural light–dark cycles with food and water ad libitum.

2.2. Surgical procedure

Rats were anesthetized with 2% isoflurane in a mixture of air and oxygen and intrathecal catheter was surgically implanted (Durect Corporation CA, USA) as described by Yaksh and Rudy (1976). The muscle attached to the nuchal crest was detached and cisternal membrane was exposed. A small cut in the membrane was made. Catheter made of 28 G polyurethane (8.5 cm long, 0.36 mm Outer Diameter and 0.18 mm Inner Diameter) was then pushed gently beneath the membrane and glided slowly towards tail. The caudal end of the catheter was placed close to the lumbar enlargement and the out-dwelling end was plugged with a piece of metal wire. After recovery, catheterized rats were subjected to stepping, placing and righting reflex tests and those exhibiting motor deficits like paralysis of hind paw were excluded from the study. Post-mortem catheter placement in the vicinity of lumbar enlargement was examined by injection of 20 μl dye (cresyl violet) into the catheter.

2.3. Drugs

Morphine sulphate (Verve Health Care Ltd. Pune, India) was diluted with normal saline. Loperamide hydrochloride and nefiracetam (Sigma-Aldrich Chemicals Ltd, MO, USA) were dissolved in vehicle made of polyethylene glycol, saline and ethanol in a ratio of 2:2:1 as described previously (Ray et al., 2005). CTOP (Sigma-Aldrich Chemicals Ltd, MO, USA) was dissolved in physiological saline.

2.4. Hargreaves plantar test

Thermally-evoked nociception to radiant heat was assessed using paw-withdrawal apparatus (UGO Basile, Italy). The rats were placed in Perspex chambers on a glass plate and radiant heat source was focused directly under the plantar surface near the heel of the rat hind paw. The resulting paw withdrawal latency (PWL) was automatically recorded on a digital screen and noted. Behaviors such as paw withdrawal and licking were recorded. The radiant heat source was adjusted to keep the baseline latencies between 8 s and 11 s. A cut-off time of 20 s was preset to prevent possible tissue damage.

2.5. Drug administration protocol

In separate groups of animals, 1 μg, 3 μg, 10 μg and 30 μg of morphine or loperamide were injected in a volume of 10 μl followed by 10 μl physiological saline (0.9%). A manual micro injector syringe (Micro-Syringe, Stoelting USA) was used for all injections after light immobilization of rats using a piece of cloth. Following drug administration, rats were subjected to Hargreaves paw withdrawal test at 15 min, 30 min, 60 min, 90 min and 120 min time points. In another set of experiment, the total duration of analgesia after single intrathecal administration of loperamide (30 μg) and morphine (30 μg) was compared.

2.6. Dose–response experiment and determination of ED_{50}

Loperamide and morphine were injected intrathecally in increasing doses (1 μg, 3 μg, 10 μg and 30 μg). Since, our preliminary experiments showed maximum analgesic effect of morphine and loperamide at 15 min and 30 min, respectively, log–dose response curves for morphine and loperamide were drawn at 15 min and 30 min, respectively, after administration. Therefore, PWLs at these time points were chosen for log–dose response relationship and their ED_{50} values were calculated. Slope of the curves were determined to get respective ED_{50}.

2.7. Reversal of analgesia

The highest dose (30 μg) of morphine and loperamide was chosen for reversal study. To study mu-opioid receptor mediated action of loperamide, CTOP was administered 10 min before loperamide and PWLs were noted 30 min after loperamide administration. For reversibility with nefiracetam, 30 nM nefiracetam (Rashid and Ueda, 2002) was administered 10 min before morphine or loperamide and these rats were subjected to Hargreaves test 30 min after morphine or loperamide administration. PWLs of CTOP alone and nefiracetam alone treated groups were also recorded.

2.8. Statistical analysis

Data are expressed as mean ± standard error of mean (S.E.M.). The data were converted to the percentage of maximal possible effect (%MPE=[(test latency-baseline latency/cut-off time -baseline latency) × 100]). Statistical analyses were done using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests to determine difference between groups at each time point. ED_{50} is reported as geometric means accompanied by their respective 95% Confidence Intervals (CI) and was calculated by linear regression analysis. Data were analysed using prism 5 (GraphPad Software, San Diego, CA, USA). A $P < 0.05$ was considered significant.

3. Results

3.1. Analgesic effect

Different groups of rats were administered loperamide and morphine in doses of (1 μg, 3 μg, 10 μg and 30 μg) intrathecally. Morphine (Fig. 1A) and loperamide (Fig. 1B) showed dose-dependent analgesic effect as compared to saline treated group. 1 μg morphine produced significant analgesic effect only at 30 min post-administration while 1 μg loperamide showed significant analgesic effect at 15 min, 30 min and 60 min compared to saline group. Vehicle treated group did not show any analgesia (data not shown). Dose to dose comparison between loperamide and morphine did not show significant difference at any time point. Morphine and loperamide showed maximum analgesic effect at 15 min and 30 min, respectively, post-administration. Therefore, effect of different doses (1 μg, 3 μg, 10 μg and 30 μg) of morphine (Fig. 2A) and loperamide (Fig. 2B) were drawn at these time points. Log–dose relationship (Fig. 3) did not reveal any significant difference in the analgesic effect of loperamide