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Involvement of peripheral mu opioid receptors in scratching behavior in mice

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ABSTRACT

Pruritus is a common adverse effect of opioid treatment. However, the mechanism by which pruritus is induced by opioid administration is unclear. In this study, we examined the effects of the intradermal injection of loperamide, a peripherally restricted opioid receptor agonist, on the itch sensation. When injected intradermally into the rostral part of the back in mice, loperamide elicited scratching behavior. We also examined the effects of the selective mu opioid receptor agonist [d-Ala^2, *N*-Me-Phe^4, Gly^5-ol]-enkephalin acetate (DAMGO), the selective delta opioid receptor agonist [d-Pen^{2,5}]-enkephalin (DPDPE), and the selective kappa opioid receptor agonist U-50488H on scratching behavior in mice in order to determine which subtype is involved in opioid-induced pruritus. Following intradermal injection into the rostral part of the back in mice, DAMGO elicited scratching behavior, while DPDPE and U-50488H did not. This suggests that peripheral mu opioid activation elicits the itch sensation. Next, we focused on the treatment of opioid-induced itch sensation without central adverse effects. Naloxone methiodide is a peripherally restricted opioid receptor antagonist. In the present study, naloxone methiodide significantly suppressed scratching behavior induced by loperamide and DAMGO. These findings suggest that mu opioid receptors play a primary role in peripheral pruritus and that naloxone methiodide may represent a possible remedy for opioid-induced itching.

1. Introduction

Pruritus is a subjective, unpleasant, irritating sensation that provokes the urge to scratch. The risk is markedly increased when opioids are given epidurally or intraspinally (Szarvas et al., 2003; Reich and Szepietowski, 2010). Consequently, itch is well known to be a common side effect of spinal opioid administration (Kjellberg and Tramèr, 2001; Reich and Szepietowski, 2010). Additionally, Ko et al. (2004) reported that scratching behavior is elicited by the intrathecal injection of morphine in primates. It is thought that the involvement of opioid receptors in pruritus and the scratching behavior induced by opioid administration are centrally mediated.

However, it has also been reported that pruritus as an adverse effect of opioid treatment is observed not only with central opioid administration but also with systemic administration for the treatment of chronic cancer pain (Cherny et al., 2001; Sartain et al., 2003). In addition, Bigliardi-Qi et al. (2004) reported that opioid receptors are expressed in peripheral nerve endings and keratinocytes in human skin. This indicates that the opioid receptors involved in itching sensations are peripheral as well as central.

Loperamide is a commonly used antidiarrhea drug and opioid agonist that does not cross the blood–brain barrier (Heykants et al., 1974). This suggests that loperamide may be effective in inducing peripheral opioid activation. In the present study, we explored the action of loperamide on scratching behavior in mice. In addition, to determine which receptor subtypes are involved in itch, we examined the effects of [d-Ala^2, *N*-Me-Phe^4, Gly^5-ol]-enkephalin acetate (DAMGO), a selective mu opioid receptor agonist; [d-Pen^{2,5}]-enkephalin (DPDPE), a selective delta opioid receptor agonist; and *trans*-(1S,2S)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide hydrochloride hydrate (U-50488H), a selective kappa opioid receptor agonist on scratching behavior in mice. Furthermore, we investigated the effects of naloxone, which is an opioid receptor antagonist, and naloxone methiodide, which is a peripherally restricted opioid receptor antagonist, on loperamide- and DAMGO-induced scratching behavior.

2. Materials and methods

2.1. Animals

Five-week-old male ICR mice were obtained from Japan SLC, Shizuoka, Japan. The animals were housed in an air-conditioned room with controlled temperature (24 ± 2 °C) and humidity (55% ± 15%). Food and water were given *ad libitum*. All procedures involving animals were conducted in accordance with the guidelines for animal experiments at Okayama University Advanced Science Research Center, and all procedures were licensed by the Animal Research Control Committee of Okayama University.

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2.2. Drugs

All drugs were obtained from Sigma-Aldrich, St. Louis, MO, USA. Loperamide hydrochloride was dissolved in physiological saline containing 0.5 Tween 80. DAMGO, DPDPE, U-50488H, naloxone hydrochloride, and naloxone methiodide were dissolved in physiological saline.

2.3. Evaluation of scratching behavior

Scratching behavior in mice was automatically detected and objectively evaluated using a MicroAct apparatus (Neuroscience, Tokyo, Japan), as reported previously (Yamamoto et al., 2010). A small Teflon-coated magnet (diameter, 1 mm; length, 3 mm) was inserted subcutaneously into both hind paws of mice under ether anaesthesia before the start of the experiment. It was confirmed that neither the operation nor the magnets affected mouse behavior. Each mouse with implanted magnets was placed in an observation chamber (diameter, 11 cm; height, 18 cm), which was surrounded by a circular coil. The electric current induced in the coil by the movement of the magnets implanted in the hind paws was amplified and recorded. The characteristic waves corresponding to scratching behaviors were then detected using a computer.

2.4. Induction of scratching behavior

The rostral part of the mouse back was clipped using hair clippers. On the next day, loperamide (1, 10, and 100 nmol/site), DAMGO (1, 10, and 100 nmol/site), DPDPE (1, 10, and 100 nmol/site) and U-50488H (1, 10, and 100 nmol/site) were administered by intradermal injection, without anaesthesia, in a volume of 0.05 ml. Mice were held to prevent struggling during injection. Immediately following the injection, mice were placed in a MicroAct observation chamber, and their behaviors were observed for 60 min.

2.5. Effects of drug administration

U-50488H was administered orally 60 min before the DAMGO injection. Naloxone hydrochloride (0.01, 0.03, and 0.1 mg/kg) or naloxone methiodide (0.01, 0.03, and 0.1 mg/kg) was injected subcutaneously into the rostral area at a short distance from the intradermal injection site. After 15 min, loperamide (100 nmol/site) or DAMGO (100 nmol/site) was injected intradermally in a volume of 0.05 ml. Immediately following the injection, mice were placed in the MicroAct observation chamber, and their behaviors were observed for 60 min.

2.6. Statistical analysis

All data are presented as the mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using one-way analysis of variance (ANOVA) with a Dunnett's test or Student's unpaired *t* test. A P value less than 0.05 was considered to be significant.

3. Results

3.1. Scratching behavior induced by loperamide

Loperamide produced dose-dependent scratching of the injected site and the surrounding region by the hind paws (Fig. 1A). The intradermal injection of loperamide produced significant scratching behavior at doses of 10 and 100 nmol/site. Fig. 1B shows the time course of scratching behaviors for 60 min after the injection of loperamide (100 nmol/site). Loperamide-induced scratching peaked within 10 min and almost subsided by 60 min.

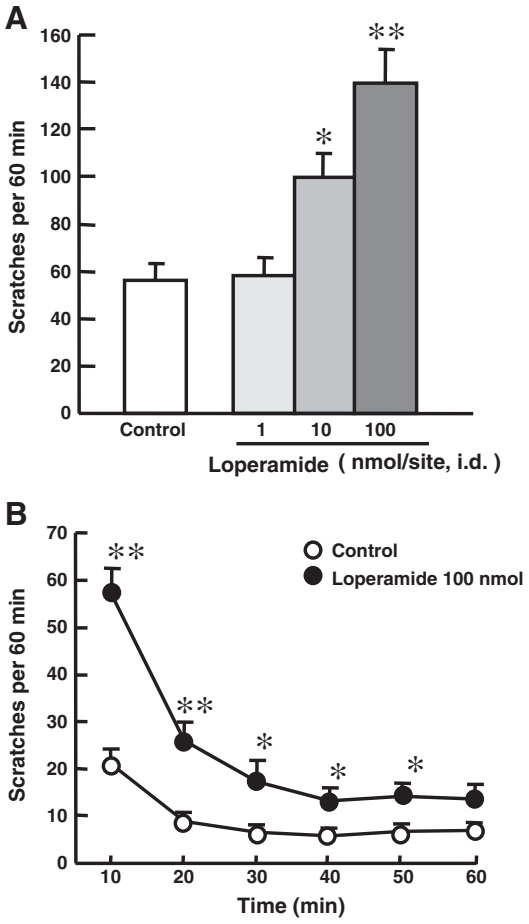

Fig. 1. Loperamide-induced scratching behavior in ICR mice. A; Dose-dependence of the scratching induced by intradermal injection of loperamide (1, 10, and 100 nmol/site) into the rostral back. B; The time course of scratching after an intradermal injection of loperamide (100 nmol/site). Each column and vertical bar represents the means ± S.E.M. (n = 10). * and **: Significantly different from the control group at $P<0.05$ and $P<0.01$, respectively (Dunnett's test and Student's unpaired test).

3.2. Scratching behavior induced by DAMGO, DPDPE, and U-50488H

DAMGO produced dose-dependent scratching of the injected site and the surrounding region by the hind paws (Fig. 2A). The intradermal injection of DAMGO produced significant scratching behavior at doses of 10 and 100 nmol/site. Fig. 2B shows the time course of scratching behaviors for 60 min after the injection of DAMGO (100 nmol/site). DAMGO-induced scratching peaked within 10 min and almost subsided by 50 min. In contrast, DPDPE and U-50488H had no effects on scratching behavior (Fig. 2C and E). Fig. 2D and F shows the time course of scratching behaviors for 60 min after the injection of DPDPE (100 nmol/site) and U-50488H (100 nmol/site), respectively. There were no significant differences compared with the saline-treated group.

3.3. Effects of U-50488H on DAMGO-induced scratching behavior

Fig. 3 shows the effects of U-50488H on DAMGO-induced scratching. Peroral treatment with U-50488H at a dose of 10 mg/kg significantly inhibited the scratching behavior induced by DAMGO (100 nmol/site).

3.4. Effects of naloxone on loperamide- and DAMGO-induced scratching behavior

Fig. 4A and B shows the effect of naloxone on loperamide- and DAMGO-induced scratching behavior. Subcutaneous pretreatment