Itch-Scratch Responses Induced by Opioids through Central Mu Opioid Receptors in Mice

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Itch • Scratch • Morphine • Opioids • Intrathecal injection • Intracisternal injection • Intradermal injection

Abstract
We examined scratch-inducing effects of intracisternal, intrathecal and intradermal injections of morphine and some opioid agonists in mice. Intracisternal injection of morphine (3 nmol/animal) and the µ-receptor agonist [D-Ala², N-Me-Phe⁴, Gly⁵-ol]enkephalin (DAMGO; 0.2 nmol/animal) elicited scratching of the face, with little effect on scratching of the trunk. Intracisternal injection of the δ-receptor agonist [D-Pen²,⁵]enkephalin (DPDPE) and the κ-receptor agonist U50488 were without effects. Intrathecal injection of morphine (0.1–3 nmol/animal) produced a dose-dependent increase in body scratching, with little effects on face scratching. Face scratching induced by intrathecal morphine (3 nmol/animal) was almost abolished by subcutaneous pretreatment with naloxone (1 mg/kg). Intradermal injections of morphine (3–100 nmol/site), DAMGO (1–100 nmol/site), DPDPE (10 and 100 nmol/site) and U50488 (10–100 nmol/site) did not elicit scratching of the site of injection. Intradermal injection of histamine (100 nmol/site) produced the scratching in ICR, but not ddY, mice and serotonin (30 and 50 nmol/site) elicited the scratching in either strain of mice. The results suggest that opioids induce scratching, and probably itching, through central µ-opioid receptors in the mouse.

Introduction
Systemic administration of morphine occasionally elicits pruritus. Morphine causes urticaria at the site of injection probably because of histamine release from mast cells, which is not mediated by opioid receptors and is not blocked by naloxone [4]. Intradermal injections of morphine, β-endorphin and FK33-824, an enkephalin analog, enhance histamine-induced itching, which is not affected by naloxone [10]. On the other hand, generalized pruritus induced by systemic butorphanol is prevented by naloxone [6]. With regard to pruritic diseases, opioid antagonists suppress itching of pruritic diseases, such as cholestasis [5, 7], chronic urticaria [17] and atopic dermatitis [17]. The plasma concentrations of enkephalins are increased in patients with cholestasis [23]. Although it is unknown whether these enkephalins act centrally to cause itching, pruritus of central origin was suggested [12]. Morphine and related opioids are administered epidurally and intrathecally to humans for pain relief. Itching is the most common adverse effect of such opioid medication [3] and pruritus is inhibited by the opioid antagonist naloxone [3, 18]. In addition, opioid antagonists suppress an experimentally induced itch in humans [8]. In animal experiments, scratching induced by intradermal injection of substance P [2] and serotonin [26] is inhibited by naloxone. Scratching is induced by morphine injection into the subarachnoid space [11, 19, 25], cisterna magna [13, 24] and medullary dorsal horn [21, 22]. Naloxone inhibits face scratching [22, 24] but not scratching induced by...
intrathecal morphine [25]. Thus, there may be several mechanisms of pruritogenic action of opioids, but the precise site(s) of the action are unclear. Therefore, in the present experiments, we compared scratch-inducing effects of intracisternal, intrathecal and intradermal injections of morphine and some opioid agonists in mice.

Materials and Methods

Animals
Male ddY or ICR mice 4–6 weeks of age were used. They were housed at a controlled temperature (23–25°C) and light (lights on from 8:00 to 20:00). Food and water were freely available.

Agents
Morphine hydrochloride (Sankyo, Tokyo, Japan), naloxone hydrochloride (Sigma, St. Louis, Mo., USA), D-Ala2, N-Me-Phe4, Gly5-ol enkephalin (DAMGO; RBI, Natick, Mass., USA), D-Pen2,5-enkephalin (DPDPE, RBI), U-50488 (trans-(+)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide) methane sulfate (RBI), serotonin hydrochloride (Sigma) and histamine (Wako Pure Chemical, Osaka, Japan) were dissolved in physiological saline. Intrathecal (via a lumbar puncture) and intracisternal injections were given in a volume of 5 µl, without anesthetizing. Intradermal injection was given in a volume of 50 µl into the rostral part of the back (around interscapular level). Naloxone (1 mg/kg) was administered subcutaneously 15 min before injection of pruritogen.

Behavioral Observation
Scratching behavior was observed as described [2, 24]. Briefly, the behavior was videotaped for 1 h with all experimenters kept out of the observation room. The video was played back to evaluate the scratching behavior. The mouse generally scratched with its hind paws several times for about 1 s and a series of these movements was counted as one bout of scratching.

Data Processing
Results are presented as the means and SE. The results were analyzed with two-way repeated measures analysis of variance (RM-ANOVA) and Dunnett's test; p < 0.05 was considered significant.

Results
The mouse (ddY strain) given an intracisternal injection of saline occasionally scratched its face and trunk with its hind paws; the number of face and body scratching per hour was 15.3 ± 4.6 and 8.3 ± 2.1, respectively (fig. 1a, b). When given an intracisternal injection of morphine (3 nmol/animal), the mouse frequently scratched its face with its hind paws: the effect peaked around 10 min and subsided by 50 min (fig. 1c). The scratching of the trunk was not increased by the same dose of morphine (fig. 1d). An intracisternal injection of the μ-opioid receptor agonist DAMGO (0.2 nmol/animal) apparently increased the scratching of the face; the effect peaked within 10 min (fig. 1e). The scratching of the trunk was not increased by the same dose of DAMGO (fig. 1f). The face scratching was not affected by the δ-opioid receptor agonist DPDPE and κ-opioid receptor agonist U50488 (fig. 1g, h). Morphine at a dose of 3 nmol/animal produced an apparent increase in locomotor activity in some mice. Other opioids at doses examined did not affect posture and gross behaviors other than scratching.

The effects of intrathecal injection of morphine into the mouse (ddY strain) on the scratching of the face and...