

## Intracisternal Injection of Opioids Induces Itch-Associated Response through $\mu$ -Opioid Receptors in Mice

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**ABSTRACT**—We examined whether opioids, especially morphine, would centrally elicit scratching in mice and determined some characteristics of the scratch-inducing action of opioids. When intracisternally (i.c.) injected, morphine (0.1–3 nmol/mouse) produced a dose-dependent increase in scratching of the face, but not of the ears, head and body trunk. When injected intradermally into the rostral part of the back, morphine (at most potent i.c. dose of 3 nmol/mouse or higher) did not increase the scratching of the injected site. Facial scratching of the mouse induced by i.c. injection of morphine (0.3 nmol/mouse) was almost abolished by distraction and by naloxone (1 mg/kg, s.c.). [D-Ala<sup>2</sup>, N-Me-Phe<sup>4</sup>, Gly<sup>5</sup>-ol]Enkephalin (DAMGO) (0.03–2 nmol), but not [D-Pen<sup>2,5</sup>]enkephalin (DPDPE) and U-50,488, dose-dependently elicited facial scratching by i.c. injection. These results suggest that morphine and DAMGO increased facial scratching, probably mediated by central opioid  $\mu$ -receptors in mice, and such scratching was due to a sensation, probably itching. The present animal model may be useful for analyzing opioid-mediated central itching.

**Keywords:** Opioid, Scratching, Itch, Intracisternal injection,  $\mu$ -Opioid receptor

Opioids, including morphine, are administered epidurally and intrathecally in humans for pain relief. Itching is the most common side effect of opioid analgesics (1–3), but not narcotic agonist-antagonist analgesics (4, 5). The opioid-induced pruritus is inhibited by the opioid antagonist naloxone (3, 6). Regarding pruritic diseases, the pruritus of cholestasis is ameliorated by opioid antagonists (7–9). Plasma concentrations of endogenous opioid peptide enkephalins are increased in patients with primary biliary cirrhosis (8). Although it is unclear whether these enkephalins act centrally to induce itch, pruritus of central origin was suggested (10) as no histamine-associated responses are observed on the skin of patients with cholestasis and their pruritus is not suppressed by anti-histamines (11, 12). Thus, opioids might induce an itch sensation centrally through opioid receptors and be involved in itch of some pruritic diseases, although their precise roles and receptor subtypes involving in the effect are still unknown. To study opioid-mediated central mechanisms of an itch sensation, animal experiments are required.

As regard to animal experiments, Koenigstein (13)

mentioned that when injected into the cisterna magna, morphine produced scratching, a possible motor response to itch, in several species including the cat, dog, rabbit and guinea pig. Microinjection of an opioid agonist into the medullary dorsal horn produces facial scratching in monkeys (14) and rats (15). These findings raise the possibility that opioids centrally induce an itch sensation in animals. However, removal of the cerebral cortex and midbrain of the cat was reported not to suppress the scratch movement induced by an intracisternal (i.c.) injection of morphine (13), a finding contrary to the idea that morphine-induced scratch is due to an itch sensation. Thus, it is still unclear whether opioids centrally induce itch in animals. As a step in assessing such a possibility, in this study we examined whether opioids, especially morphine, could centrally elicit scratching in mice and determined some characteristics of the scratch-inducing action of opioids, which involve opioid receptor subtypes.

### MATERIALS AND METHODS

#### Materials

Morphine hydrochloride (Sankyo, Tokyo), naloxone hydrochloride (Sigma, St. Louis, USA), [D-Ala<sup>2</sup>, N-Me-

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Phe<sup>4</sup>, Gly<sup>5</sup>-ol]enkephalin (DAMGO; RBI, Natick, MA, USA), [D-Pen<sup>2,5</sup>]enkephalin (DPDPE, RBI) and U-50,488 (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide) methane sulfate (RBI) were dissolved in physiological saline. Opioids agonists were intracisternally (i.c.) injected in a volume of 5 µl without incision and anesthetizing. In case of intradermal injection, morphine was injected into the rostral part of the back in a volume of 50 µl. Naloxone (1 mg/kg) was s.c. injected into the back 15 min before morphine injection.

#### Behavioral experiments

Male ddY mice (4- to 5-weeks-old) weighing 23–27 g (average of 24.5 g) were used. They were housed under controlled temperature (23–25°C) and light (lights on from 08:00 to 20:00). Food and water were freely available. Before experiment, the animals were put into an acrylic cage (13 × 9 × 30 cm) for 1 hr for acclimation. Immediately after opioid injection, they were put back into the same cage and their behaviors were recorded by an 8-mm video camera (CCD-TRV 60; Sony, Tokyo), unless otherwise mentioned, under unmanned conditions, which served for the observation of scratching. Each mouse was used for only one experiment. The mice generally scratched themselves several times for about 1 sec and a series of such movements was counted as one incidence of scratching. Since higher doses of morphine and DAMGO increased locomotion activity and decreased scratching behavior of the mouse, scratching data from mice that showed an apparent increase in locomotion activity was discarded.

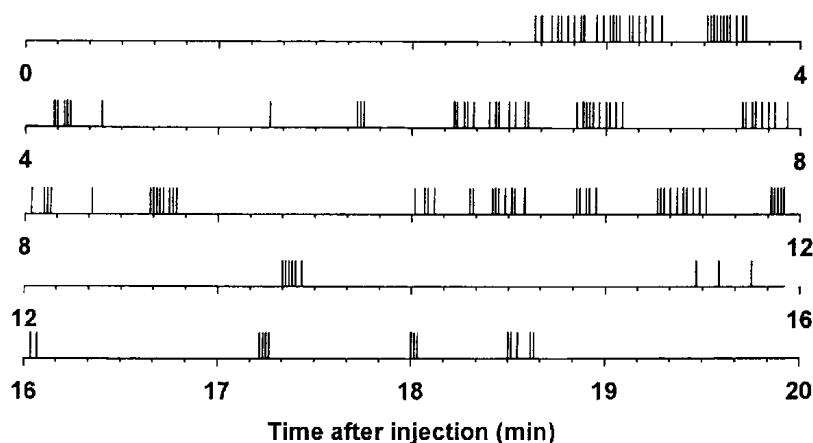
#### Data processing

Statistical comparisons were made by repeated meas-

ures two-way analysis of variance (RM-ANOVA) or one-way ANOVA and *post hoc* Dunnett's multiple comparisons;  $P < 0.05$  was considered significant. The means of data are presented together with S.E.M.

#### RESULTS

When given an i.c. injection of morphine (3 nmol/mouse), the mouse frequently scratched its face by the hind paws, which is shown in Fig. 1 as an example. In this case, the scratching was 2.6 min in onset and appeared intermittently for at least 30 min (a period of 20 min is shown in Fig. 1). An i.c. injection of morphine at doses of 0.1–3 nmol/mouse produced a dose-dependent increase in facial scratching (Fig. 2: A–D); RM-ANOVA analysis revealed a significant morphine effect ( $F_{3,32} = 4.93$ ,  $P < 0.01$ ) and morphine × time interaction ( $F_{15,160} = 3.17$ ,  $P = 0.001$ ). As compared to the saline control, an increase in scratching was apparent at doses of 0.3 and 3 nmol/mouse. Following these doses, the median values of onset of the scratching were 4.6 ( $n = 9$ ) and 4.2 ( $n = 8$ ) min at 0.3 and 3 nmol/mouse, respectively; the effect peaked around 10 min and subsided by 50 min. There were no apparent alterations in gross behaviors and posture following doses of 0.3 nmol/mouse or less, while 5 out of 13 animals showed an apparent increase in locomotion activity following a dose of 3 nmol and their data was discarded. A higher i.c. dose of morphine (30 nmol/mouse) produced an apparent increase in locomotion activity in 8 out of 15 animals and decreased facial scratching even in 7 animals with no apparent alterations in gross behaviors and posture (Fig. 2E). These i.c. doses of morphine (0.1–30 nmol/mouse) did not increase scratching of the ears, head and body trunk (Fig. 2E) and



**Fig. 1.** An example of facial scratching following intracisternal injection of morphine into the mouse. The mouse was given 3.0 nmol/mouse of morphine. Each bar represents one incidence of facial scratching by the hind paw, generally several scratching behaviors for about one second.

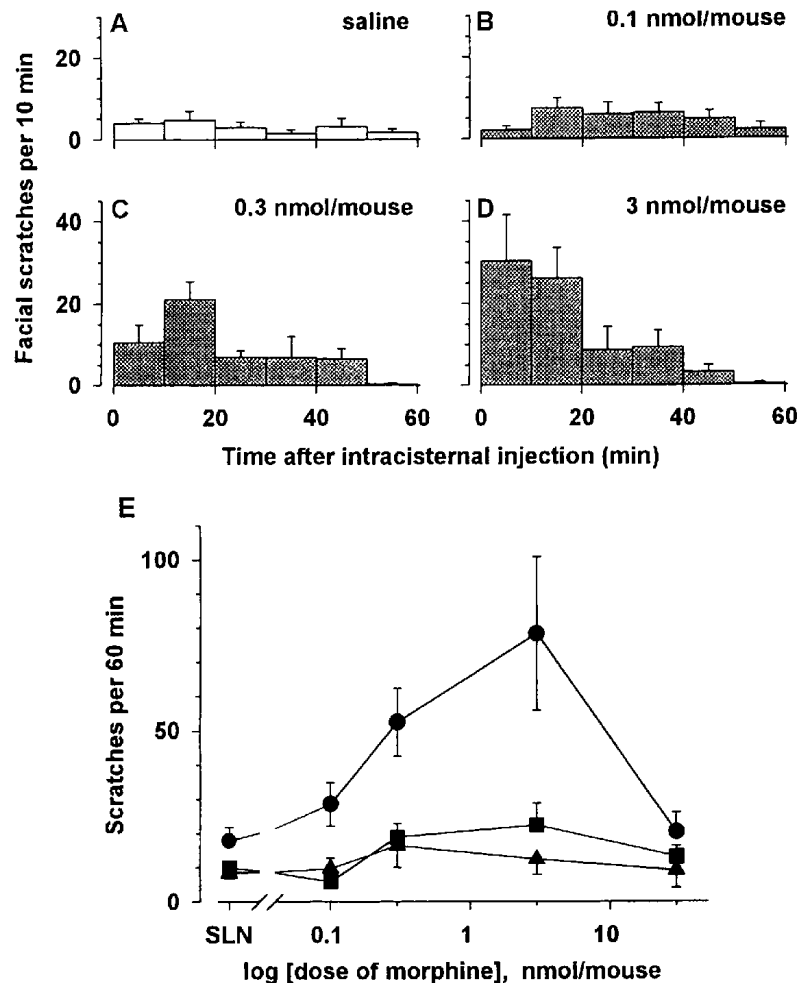


Fig. 2. Time course of facial scratching after intracisternal injection of morphine (A–D) and dose-response curves for scratch-inducing effects of intracisternal injection of morphine (E). Data from 10 (A–D)- or 60 (E)-min periods are presented as means and S.E.M. from 7–10 mice. Scratching regions: face (●), body trunk (▲), ears and head (■).

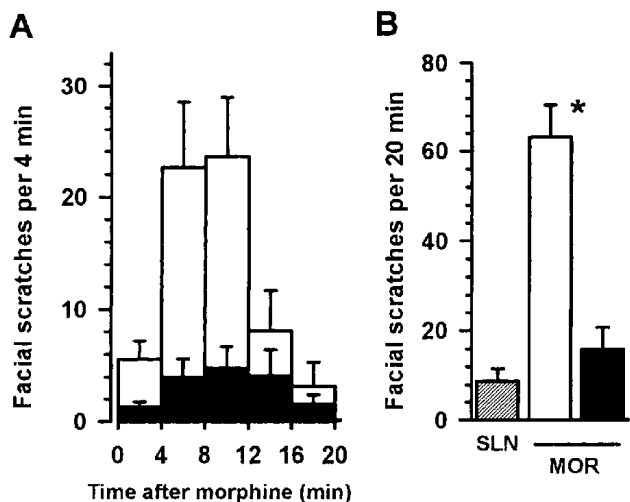
behaviors other than scratching such as grooming and forelimb motion (data not shown). In the following experiments, we used an i.c. morphine dose of 0.3 nmol/mouse that did not produce any apparent alterations in locomotion activity in all animals tested.

To determine whether morphine could peripherally act to induce scratching, we examined the effect of intradermal injection of morphine. When injected intradermally into the rostral part of the back, morphine (at most potent i.c. dose of 3 nmol/mouse or higher) did not increase the scratching of the injected site; scratches following saline or morphine at doses of 3, 30 and 100 nmol/mouse were  $14.0 \pm 4.1$ ,  $18.8 \pm 3.6$ ,  $17.5 \pm 3.0$ , and  $21.1 \pm 4.8$  per 60 min ( $n=8$  each group), respectively.

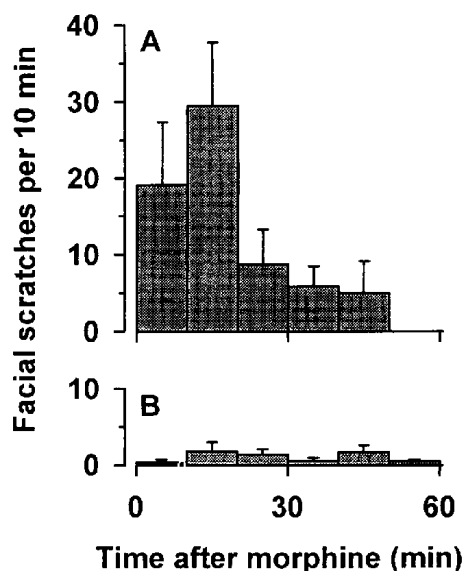
An itch sensation is profoundly affected by psychological modification and easily suppressed by a distraction task (16). Therefore, if the facial scratching following i.c. morphine is due to an itch sensation, it will be suppressed

by distraction. On the contrary, if morphine acts on the motor (scratch reflex) center to induce scratching, it will not be suppressed by distraction. Thus, we examined the effect of distraction (attracting the attention of the mouse to the experimenter) on morphine-induced scratching. As compared to control scratching under circumstances without humans in the laboratory, scratching induced by morphine (0.3 nmol/mouse, i.c.) was markedly inhibited by distraction at every period in the time course (Fig. 3A); RM-ANOVA revealed a significant effect of distraction ( $F_{1,19}=19.52$ ,  $P<0.001$ ) and distraction  $\times$  time interaction ( $F_{4,76}=3.10$ ,  $P<0.05$ ). As shown in Fig. 3B, morphine (0.3 nmol/mouse) significantly increased the facial scratching, and the morphine-induced scratching was almost completely inhibited by distraction.

Itching of humans given intrathecal or epidural opioids is inhibited by opioid antagonists (3, 6). On the other hand, agitation responses, including scratching of the



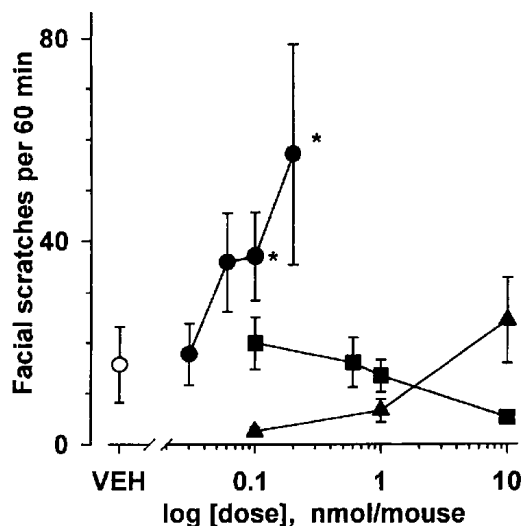
**Fig. 3.** The effect of distraction on the facial scratching induced by morphine injected into the cisterna magna of the mouse. Immediately after the intracisternal injection of morphine (0.3 nmol/mouse), the mouse behavior was videotaped in the presence of the experimenter (for producing distraction, closed columns,  $n=11$ ) or in his absence (control, open columns,  $n=9$ ) beside the animals. Values represent the number of facial scratchings per 4 min (A) or 20 min (B) as means and S.E.M. In panel B, SLN represents an intracisternal injection of saline (a hatched column,  $n=9$ ), and MOR represents an intracisternal injection of morphine (0.3 nmol/mouse) with (a closed column) or without (an open column) distraction. \* $P<0.05$ , when compared with saline (SLN).



**Fig. 4.** The effect of naloxone on the facial scratching induced by intracisternal morphine injection in mice. The mice were given an s.c. injection of saline ( $n=11$ ) (A) or naloxone (1 mg/kg,  $n=10$ ) (B) and 15 min later, an intracisternal injection of morphine (0.3 nmol/mouse). Values represent means and S.E.M.

skin, of the rat induced by a high intrathecal dose of morphine are not inhibited by the opioid antagonist naltrexone (17). Thus, we examined the effect of pretreatment with naloxone on the scratch-inducing action of i.c. morphine. Pretreatment with naloxone (1 mg/kg, s.c.) almost abolished the scratching induced by an i.c. injection of morphine at a dose of 0.3 nmol/mouse (Fig. 4); RM-ANOVA analysis of data between 0 and 50 min after injection revealed a significant naloxone effect ( $F_{1,19}=8.99$ ,  $P<0.01$ ) and treatment  $\times$  time interaction ( $F_{4,76}=3.68$ ,  $P<0.01$ ).

To determine which of the opioid  $\mu$ -,  $\delta$ - and  $\kappa$ -receptor subtypes would be involved in scratching following i.c. injection, we examined the effects of three receptor subtype-selective agonists. An i.c. injection of DAMGO, a selective  $\mu$ -receptor agonist (18), at doses of 0.03–0.2 nmol/mouse produced a dose-dependent increase in facial scratching without other apparent behavioral alterations (Fig. 5). Following a dose of 0.2 nmol/mouse, scratching was 2.4 min in median value of onset, peaked at the initial 10-min period, and subsided in 40 min ( $n=7$ ). Injection of DAMGO at higher i.c. doses of 0.3 and 1 nmol/mouse produced an apparent increase in locomotion activity in 4 of 4 and 7 of 12 animals, respectively. An i.c. injection of DPDPE, a selective  $\delta$ -receptor agonist (19), at doses of 0.1–10 nmol/mouse did not significantly increase facial scratching (Fig. 5) without apparent alterations in gross behaviors, except of slight extension of the extremities at the highest dose examined.



**Fig. 5.** Effects of intracisternal injections of agonists selective for opioid receptor subtypes on facial scratching in mice. The mouse was given an intracisternal injection of DAMGO (closed circles,  $n=7-8$ ), DPDPE (closed triangles,  $n=8$ ), U-50,488 (closed squares,  $n=7-15$ ) or saline (VEH, an open circle,  $n=11$ ). Facial scratches per 60 min were plotted against the dose. Values represent means and S.E.M. \* $P<0.05$ , when compared with saline (VEH).

An i.c. injection of U-50,488, a selective  $\kappa$ -receptor agonist (20), at doses of 0.1–10 nmol/mouse also did not significantly increase facial scratching without apparent alterations in gross behaviors.

## DISCUSSION

In the present report, we demonstrated that an i.c. injection of morphine into the mouse produced a dose-dependent increase in scratching of the face. A dose as low as 0.3 nmol/mouse was effective. Higher i.c. doses of morphine (3 and 30 nmol/mouse) and DAMGO (0.3 and 1 nmol/mouse) produced increases in locomotion activity. Given that the body weight was 25 g, 0.3 nmol/mouse of morphine corresponds to about 0.005 mg/kg, which was about 150 times less than the i.c. dose of morphine (0.7 mg/kg) reported to produce facial scratching in several animals including cats (13). In human subjects, epidural injection of morphine at doses of 2–5 mg (corresponding to 0.04–0.1 mg/kg if body weight was 50 kg) was claimed to produce itching (1, 21, 22). Although it is difficult to compare pruritogenic doses of morphine between intracisternal (mouse, in the present experiments) and epidural injections (human), the former may be comparable to the latter.

Facial scratching of the mouse induced by i.c. injection of morphine (0.3 nmol/mouse) was almost abolished by naloxone, suggesting mediation of opioid receptors. Morphine is a relatively selective to opioid  $\mu$ -receptor (23) and facial scratching was also induced by another  $\mu$ -receptor agonist DAMGO. The onset and peak time of the scratching were not quite different between morphine and DAMGO, suggesting same acting sites of these drugs. On the other hand, the  $\delta$ -receptor agonist DPDPE and  $\kappa$ -receptor agonist U-50,488 were without effects. These results suggest that facial scratching induced by i.c. injection of opioids is primarily mediated by opioid  $\mu$ -receptors, but not by opioid  $\delta$ - and  $\kappa$ -receptors.

Because morphine acts on mast cells to release the pruritogenic chemical mediator histamine (24, 25), it may be conceivable that following i.c. injection, morphine acted on the skin to elicit scratching. However, morphine at doses of 0.3 and 3 nmol/mouse apparently increased facial scratching following i.c. injection, while the same or higher doses of morphine did not increase scratching after intradermal injection into the rostral back, a region that is sensitive to the pruritogenic agent compound 48/80 in producing scratching (26). Therefore, it is likely that i.c.-injected morphine acted centrally, rather than peripherally, to elicit scratching.

Opioid  $\mu$ -receptor agonists, but not  $\delta$ - and  $\kappa$ -receptor agonists, elicited facial scratching following i.c. injection in mice (present experiments) and injection into the

medullary dorsal horn in monkeys (14). In addition, facial scratching following opioid injection either into the cisterna magna (present experiments) or into the medullary dorsal horn (14, 15) was naloxone-reversible. These findings taken together suggest that common neural systems are involved in scratch-inducing actions of opioids injected into the cisterna magna and the medullary dorsal horn. However, the time course of these opioid actions is slow and peaks at 30–40 min after the injection into the medullary dorsal horn (15), whereas the scratching peaked around 10 min after i.c. injection of opioids in the present experiments. These observations may show that the primary site of opioid actions is not necessarily the medullary dorsal horn.

Although itch is a sensation associated with a desire to scratch, it is difficult to determine whether scratching induced by opioids injected into the central nervous systems would be due to an itch sensation (13, 17). Facial scratching behavior induced by morphine at an i.c. dose of 0.3 nmol/mouse was markedly suppressed by distraction, features similar to an itch sensation in humans (16, 27), findings suggesting that such facial scratching is due to a sensation rather than to direct motor effect. Facial scratching induced by morphine (0.3 nmol/mouse, i.c.) was suppressed by naloxone. As mentioned above, itching following epidural morphine (3, 6) and the pruritus of cholestasis (7–9) is inhibited by naloxone. Thus, the present results are consistent with the view that facial scratching induced by i.c. morphine, at least at a dose of 0.3 nmol/mouse, is due to an itch sensation.

In summary, we demonstrated that i.c. injection of relatively low doses of morphine and DAMGO increased facial scratching, probably mediated by central opioid  $\mu$ -receptors in mice and suggest the possibility that such scratching was due to a sensation, probably itching, at least after an i.c. dose of 0.3 nmol/mouse. This may be a useful animal model of an opioid-mediated central itching.

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