Itch-Associated Response and Antinociception Induced by Intracisternal Endomorphins in Mice

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ABSTRACT—Endomorphin-1 and endomorphin-2 are newly identified endogenous peptides and have high affinity and selectivity for µ-opioid receptors. The present experiments were conducted to determine whether intracisternal injection of these peptides would produce an itch-associated response and antinociception and to compare their effects to that of morphine. Endomorphin-1 and endomorphin-2 (0.3–3 nmol/mouse) elicited facial scratching characterized by bell-shaped dose-response curves with a peak effect at endomorphin-1 at 0.3 nmol/mouse and endomorphin-2 at 1 nmol/mouse. Their peak effects were inhibited by subcutaneous pretreatment with naloxone (1 mg/kg). Morphine (0.3–30 nmol/mouse) produced facial scratching, and its dose-response curve was also bell-shaped. Scratching of the body trunk, head and ears were not elicited by these doses of endomorphins and morphine. Endomorphin-1 and -2 at doses of 0.3–3 nmol/mouse produced dose-dependent antinociception, as measured with the tail-pressure test. The potency and duration of actions of these peptides were comparable to those of morphine. The results suggest that endomorphin-1 and endomorphin-2 are involved in itch-signaling and pain-inhibiting functions of the brain.

Keywords: Endomorphin-1 and -2, Facial scratching, Itch, Analgesia, Intracisternal injection

Systemic administration of morphine occasionally causes pruritus, which is probably due to histamine released from the mast cells and may not be mediated by opioid receptors (1). On the other hand, when morphine is administered epidurally or intrathecael, the most common side effect is pruritus, which is generalized or localized to the face, neck and upper thorax, with the onset after 30 min to a few hours (for reviews, see refs. 2 and 3). Opioid antagonists block pruritus induced by central injection of morphine (4). In addition, the opioid antagonists inhibit experimentally induced itching (5) and suppress an itch sensation (and scratching) of patients with chronic cholestasis, chronic urticaria or atopic dermatitis (6–8). In animal experiments, morphine elicits facial scratching after intracisternal (i.c.) injection in mice (9) and injection into the medullary dorsal horn of rats (10) and monkeys (11). Morphine is relatively selective for µ-opioid receptors, and facial scratching is also induced by i.c. or intramedullary injection of the µ-opioid receptor agonist [D-Ala², N-Me-Phe⁴, Gly⁵-ol]enkephalin (DAMGO), but not by agonists for δ- or κ-opioid receptors (9, 11). These findings taken together raise the possibility that µ-opioid receptors and endogenous ligands for µ-receptors are involved in central processing of the itch sensation. However, as several opioid peptides have a greater or lesser degree of affinity for µ-opioid receptors (12), it is unknown which opioid peptides are involved in such a function.

Endomorphin (EM)-1 and EM-2 are endogenous peptides recently isolated from bovine frontal cortex (13). These peptides were also identified in the human brain (14), and EM-2-like immunoreactivity is heterogeneously distributed in the rat brain (15). They have high affinity and selectivity for µ-opioid receptors (13), and they are partial agonists for µ-opioid receptors, as assessed using a G protein activation system (16, 17). Endomorphins were shown to produce antinociceptive action after intracerebroventricular and intrathecal injection, and their potencies were as high as those of morphine and DAMGO (13, 18). However, the antinociceptive effects of

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i.c. injection of endomorphins remain unknown. Therefore, we conducted the present experiments, using morphine as control opioid, to determine whether EM-1 and EM-2 would elicit itch-associated response (scratching) after i.c. injection and to compare their scratch-inducing effects with the antinociceptive ones.

MATERIALS AND METHODS

Animals
Male ddY mice (Japan SLC, Hamamatsu) of 4–5 weeks of age were used. They were housed under controlled temperature (23–25°C) and light (light from 7:00 to 19:00). Food and water were freely available.

Agents
EM-1 and EM-2 (synthesized by K.K. or purchased from Peptide Institute, Mino, Osaka), morphine hydrochloride (Sankyo, Tokyo) and naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in physiological saline. EM-1, EM-2 and morphine were injected i.c. in a volume of 5 μl, as described (9). Naloxone was injected subcutaneously 15 min before i.c. injection. Dosages are given in terms of mol or the weight of the respective salt.

Observation of scratching
Scratching behaviors were observed as described (9). Briefly, the behaviors of the mouse were videotaped without any experimenter in the observation room, and the play-back of the video was used for counting scratching.

fig. 1. An example of facial scratching after an i.c. injection of EM-1. EM-1 at a dose of 0.3 nmol/mouse was injected at time 0. Each bar represents a series of facial scratching for about 1 sec.

fig. 2. Time course of facial scratching after i.c. injection of endomorphins and morphine. The mice were given EM-1 (0.3 nmol/mouse, n=7) (A), EM-2 (1 nmol/mouse, n=7) (B) or morphine (3 nmol/mouse n=8) (C). Values represent the mean of facial scratches per 5 min together with S.E.M.

Analgesic test
Analgesic experiments were conducted according to the guidelines published in a Guest Editorial in Pain on ethical standards for investigations of experimental pain in animals (19). The nociceptive sensitivity of the mouse was determined with the tail-pressure test (20), using a pressure analgesimeter (Ugo Basile, Milan, Italy) with a wedge-shaped piston. Pressure stimulation was applied to the mouse tail at a loading rate of 32 g per sec, and the pressure eliciting struggle behavior was determined as the nociceptive threshold.
Data processing

The means of data are presented together with S.E.M. Results were analyzed with two-way analysis of variance (ANOVA), two-way repeated measures analysis of variance (RM-ANOVA) or Student’s t-test; a P < 0.05 value was considered significant.

RESULTS

Itch-associated responses

Figure 1 shows an example of facial scratching after i.c. injection of EM-1 (0.3 nmol/mouse). The mouse showed the first facial scratching 6 min 24 sec after the injection and then intermittent scratching. High frequency of scratching was observed between 6 and 9 min after injection and almost subsided by 14 min. Figure 2 shows the time course of facial scratching after i.c. injections of EM-1 (0.3 nmol/mouse), EM-2 (1 nmol/mouse) and morphine (3 nmol/mouse). The median onset of facial scratching after EM-1 (0.3 nmol/mouse) was 6.0 min (n = 7), and the effect peaked between 5 and 10 min after injection and almost subsided by 25 min (Fig. 2A). The median onset of facial scratching after EM-2 (1 nmol/mouse) was 3.6 min (n = 7). Although the time course of EM-2 action was relatively flat, it peaked between 10 and 15 min and almost subsided by 40 min (Fig. 2B). The median onset of facial scratching after morphine (3 nmol/mouse) was 4.6 min (n = 8), and the effect peaked between 5 and 10 min after injection and almost completely subsided by 40 min (Fig. 2C).

EM-1 at i.c. doses of 0.03 to 3 nmol/mouse apparently elicited facial scratching, with little effect on scratching of the body trunk, ears and head (Fig. 3A); ANOVA revealed a significant effect on the face (P < 0.0001). The dose-response curve was bell-shaped with a peak effect after 0.3 nmol/mouse (Fig. 3A). EM-2 at i.c. doses of 0.3 to 3 nmol/mouse also produced facial scratching, with little effect on scratching of the body trunk, ears and head (Fig. 3B); ANOVA revealed a significant effect on the face (P < 0.0001). The dose-response curve was also bell-shaped with a peak effect after 1 nmol/mouse (Fig. 3B). Morphine at i.c. doses of 0.1 to 3 nmol/mouse produced dose-dependent facial scratching, but a higher dose of 30 nmol/mouse was almost without effect (Fig. 3C); ANOVA revealed a significant effect on the face (P < 0.0001). Morphine at all doses examined was with little effect on scratching of the body trunk, ears and head (Fig. 3C). Apparent changes in gross behaviors other than scratching and apparent increase in locomotor activity were not observed after EM-1 or EM-2, at the doses examined. Loco-

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**Fig. 3.** Dose-response curves for scratch-inducing effects of i.c. injection of endorphins and morphine. The mice were given EM-1 (A), EM-2 (B) or morphine (C); and scratching of the face (●), body trunk (▲) or ears and head (■) was counted for 60 min. Each point represents the mean and S.E.M. of 7–10 animals. ANOVA: A: Effect of region, F(2,102) = 35.45, P < 0.0001. B: Effect of region, F(2,105) = 33.41, P < 0.0001; effect of dose, F(4,105) = 4.36, P < 0.01; dose x region interaction, F(8,105) = 3.27, P < 0.01. C: Effect of region, F(2,141) = 31.19, P < 0.0001; effect of dose, F(5,141) = 5.53, P < 0.001; dose x region interaction, F(10,141) = 2.45, P = 0.01.
motor activity was not apparently affected by morphine at doses of 0.1–3 nmol/mouse, but a marked increase was observed after the higher dose of 30 nmol/mouse. Subcutaneous pretreatment with naloxone (1 mg/kg) significantly suppressed facial scratching induced by i.c. injection of EM-1 (0.3 nmol/mouse) and EM-2 (1 nmol/mouse) (Fig. 4); the number of facial scratching of the naloxone-pretreated mouse in either the EM-1 or EM-2 group was similar to that of the mouse given i.c. saline (Fig. 3A).

**Antinociceptive effects**

EM-1 at i.c. doses of 1 and 3 nmol/mouse produced dose-dependent antinociceptive effects, without apparent effect after 0.3 nmol/mouse (Fig. 5A). EM-2 at i.c. doses of 1 and 3 nmol/mouse also produced dose-dependent antinociceptive effects, with little effect at 0.3 nmol/mouse (Fig. 5B). Morphine at an i.c. dose of 3 nmol/mouse produced marked antinociception, although lower doses of 0.3 and 1 nmol/mouse were without apparent effects (Fig. 5C). The effects of EM-1, EM-2 and morphine peaked between 15 and 30 min after injection and subsided by 2 hr (Figs. 5 and 6). These opioids exhibited similar dose-response relationships (Fig. 5D). The antinociceptive effects of EM-1 (3 nmol/mouse) and EM-2 (3 nmol/mouse) were both significantly inhibited by pretreatment with naloxone (Fig. 6).

![Fig. 4. Suppression by naloxone of facial scratching induced by i.c. injection of endomorphins. The mice were given an i.c. injection of EM-1 (0.3 nmol/mouse) or EM-2 (1 nmol/mouse) 15 min after pretreatment with subcutaneous injection of saline (open columns) or naloxone (1 mg/kg; hatched columns). Values represent the number (mean and S.E.M.) of facial scratching per 60 min. n=14–16. *P<0.05, when compared with the corresponding control (Student’s t-test).](image)

![Fig. 5. Antinociceptive effects of i.c. injection of endomorphins and morphine. A–C: Time course of antinociceptive effects of EM-1 (A), EM-2 (B) and morphine (C). D: Dose-response curves for EM-1, EM-2 and morphine. The mice were given i.c. injection of saline (○), EM-1, EM-2 or morphine at doses of 0.3 (●), 1 (▲) or 3 (▲) nmol/animal. The nociceptive threshold of each animal determined immediately before injection served as a control (100%). D: The peak response is plotted on the ordinate against the log dose on the abscissa. Values are the means and S.E.M. of 7–10 animals. RM-ANOVA: A: Main effect of EM-1, F(3,27)=17.5, P<0.0001; dose×time interaction, F(18,162)=10.8, P<0.0001. B: Main effect of EM-2, F(3,32)=3.22, P<0.05; dose×time interaction, F(18,192)=4.03, P<0.0001. C: Main effect of morphine, F(3,30)=12.08, P<0.0001; dose×time interaction, F(18,180)=9.99, P<0.0001.](image)
the incidence of pruritus is low after the antagonist analgesics, butorphanol and buprenorphine (21), suggesting the importance of μ-opioid receptors in central itching.

One important finding in our study is that i.c. injections of EM-1 (0.3 nmol/mouse) and EM-2 (1 nmol/mouse) elicited facial scratching and that their potencies were comparable to that of morphine. The effects of EMs were markedly suppressed by naloxone, suggesting the involvement of opioid receptors. Since EM-1 and EM-2 have high affinity and selectivity for μ-opioid receptors (13) and EM-2 was demonstrated to produce central effects mainly through μ-opioid receptors (22), EMs may induce the itch-associated response through μ-opioid receptors. EM-2-like immunoreactivity is distributed in the rat brain (15). With these findings taken into account, the present results raise the possibility that EM-1 and/or EM-2 are involved in central itch processing.

Although morphine at i.c. doses of 0.3 and 1 nmol/mouse did not affect the nociceptive threshold, it produced apparent facial scratching. Similarly, although the antinociceptive effects of EM-1 (0.3 nmol/mouse) and EM-2 (1 nmol/mouse) were negligible and slight, respectively, they produced a maximum scratching effect. Thus, the facial scratch-inducing doses of EMs and morphine were lower than the antinociceptive doses. In addition, the scratch-inducing effects of EMs and morphine peaked more rapidly than the antinociceptive effects. The effects of EM-1 (0.3 nmol/mouse), EM-2 (1 nmol/mouse) and morphine (3 nmol/mouse) peaked during a period of 5–10, 10–15 and 5–10 min, respectively, while the antinociceptive effects of EMs and morphine peaked between 15 and 30 min. These findings suggest that the site of the scratch-inducing action of i.c. EMs and morphine is different from that of the antinociceptive action. Possible sites of antinociceptive action of i.c. injection of opioids are the nucleus gigantocellularis (23) and periaqueductal gray matter (24). On the other hand, the precise site of the scratch-inducing action of i.c. injected EMs and morphine is unclear. Because the median onset of facial scratching was several minutes, we could rule out the regions around the cisterna magna. Injection of morphine into the medullary dorsal horn elicits naloxone-reversible facial scratching (10, 11). This finding raises the possibility that the medullary dorsal horn is a site of action of i.c. injection of EMs and morphine. However, the peak effect of morphine injected into the medullary dorsal horn is 30–40 min after injection (10, 11), which is slower than the effect of i.c. injection of morphine (ref. 9 and the present experiment). These observations suggest that the primary site of the scratch-inducing action of i.c. opioids is not necessarily the medullary dorsal horn.

Dose-response curves for facial scratch-inducing effects

**DISCUSSION**

Intracisternal injection of morphine at doses of 0.1 to 3 nmol/mouse elicited facial scratching, although it did not significantly increase scratching of other body regions such as the body trunk, ears and head, a result confirming out previous report (9). Facial scratching induced by morphine (0.3 nmol/mouse, i.c.) is markedly suppressed by naloxone pretreatment and distraction stimulation (9). Facial scratching is also induced by i.c. injection of the μ-opioid receptor agonist DAMGO, but not the δ-opioid receptor agonist [d-Pen^1^,d-Pen^2^]enkephalin and the κ-opioid receptor agonist U-50,488 (9). Considering that intradermal injection of morphine at doses as high as 3–100 nmol/site does not elicit scratching (9), these findings suggest that opioids induce the itch-associated response through μ-opioid receptors in the brain. Similarly in humans, epidural injection of the μ-opioid receptor agonists, morphine and fentanyl, produce pruritus, while
of EM-1, EM-2 and morphine were bell-shaped. Although morphine at doses of 0.1–3 nmol/mouse did not produce any apparent increase in locomotor activity in the mouse, morphine at the higher dose of 30 nmol/mouse increased it. The potent μ-opioid receptor agonist DAMGO also increases locomotor activity after i.c. doses of 0.3 and 1 nmol/mouse (9). Therefore, although EMs at the doses examined did not produce an apparent increase in locomotor activity, it is possible that high doses of these peptides exert undetectable central locomotor effects to inhibit the facial scratching. Higher doses of EMs and morphine produced antinociceptive effects; that is, inhibition of behavioral aversive responses. Thus, another possible explanation for the bell-shaped dose-response curve is that the antinociceptive action of higher doses of these opioids was responsible for the inhibition of facial scratching. However, although EM-2 (1 nmol/mouse) and morphine (3 nmol/mouse) produced apparent antinociceptive effects, their scratch-inducing activities peaked at these doses. Therefore, the antinociceptive action may not be the primary cause of inhibition of facial scratching.

EM-1 increased the facial scratching at doses of 0.03 and 0.3 nmol/mouse, while EM-2 had little or no effect at the same doses. Thus, EM-1 was more potent than EM-2 at the low doses. The effective dose of EM-1 (0.03–0.3 nmol/mouse, present experiments) was comparable with that of DAMGO (0.06–0.2 nmol/mouse) (9). The affinity of EM-1 for μ-opioid receptors is higher than that of EM-2 and similar to that of DAMGO; the Kᵯ of EM-1, EM-2 and DAMGO is 0.36, 0.69 and 0.34 nM, respectively (13). EM-1 is more potent than EM-2 in inhibiting Ca²⁺ channel currents in μ-opioid receptor-expressing cells; the IC₅₀ for EM-1 and EM-2 is 7.7 and 23.1 nM, respectively (25). Thus, the facial scratching-inducing activity of i.c. injection of EMs corresponded to their affinity for μ-opioid receptors. Although EM-1 and EM-2 were reported to have similar μ-receptor affinities in μ-receptor-transfected B2 fibroblasts, it should be noted that their affinities were relatively low; the Kᵯ of EM-1 and EM-2 is 1, 117 and 900 nM, respectively (16).

The antinociceptive effects of EM-1 and EM-2 at an i.c. dose of 3 nmol/mouse was inhibited by naloxone, the findings suggesting that these effects were mediated by opioid receptors. The potency and duration of antinociceptive effects of i.c. injected EM-1 and EM-2 were comparable to those of i.c. morphine, the results being comparable to antinociception after intracerebroventricular injection of EMs (13). In contrast, the antinociceptive effects of intracereally injected EMs is rapid and short-lasting; the effects peak at 2 min and almost subside within 15 min after injection (18). It is obscure why the time course differs between the cerebral and spinal ac-

REFERENCES


