Intracisternal, But Not Intrathecal, Injection of Naloxone Inhibits Cutaneous Itch-Related Response in Mice

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The present study was conducted to determine whether cutaneous itch involves mu-opioid receptors in either of the spinal cord or lower brainstem or in both regions in mice. An intraplantar injection of serotonin hydrochloride (100 nmol/site) induced biting, an itch-related behavior. The behavior was inhibited by subcutaneous (0.3—1 mg/kg) and intracisternal (1—10 nmol/site), but not intrathecal (1—10 nmol/site), injections of naloxone hydrochloride. An intradermal injection of serotonin (100 nmol/site) to the rostral back induced scratching, an itch-related behavior, which was inhibited by subcutaneous (1 mg/kg) and intracisternal (10 nmol/site) injections of naloxone. These results suggest that mu-opioid receptor in the lower brainstem, but not spinal cord, is a site of central pruritogenic action of opioids and is involved in the facilitatory regulation of itch signaling.

Key words mu-opioid receptor; serotonin; itch; intracisternal; intrathecal

Mu-opioid receptor antagonists have been claimed to produce inhibitory effects on experimentally induced itch in normal subjects[1] and pruritus in patients with dermatoses and visceral diseases.[2—4] In animal experiments, mu-opioid receptor antagonists suppress itch-related behaviors in several kinds of itch models.[5—10] The mu-opioid receptor antagonist naltrexone suppresses itch-related behaviors without effects on the increased activity of primary afferents, suggesting that it inhibits itch through central action.[6]

There are at least two possible sites of pruritogenic action of opioids, the spinal dorsal horn and the lower brainstem. An intradermal injection of histamine induces hyperalgesia (enhanced prickling pain) and hyperknesis (enhanced pricking-evoked itch), and intradermal pretreatment with the local anesthetic chloroprocaine produces the inhibition of pain and the enhancement of itch.[11] In contrast, itch sensation is generally reduced by pain produced by scratching. Therefore, it was suggested that itch produced by intrathecal and epidural injection of mu-opioid receptor agonists in humans is due to the inhibition of pain input by opioids in the dorsal horn.[12]

Regarding the lower brainstem, injections of mu-opioid receptor agonists into the cisterna magna and medullary dorsal horn evoked facial scratching in animals.[13—16] Since naltrexone inhibits scratching, but not Fos expression in the spinal dorsal horn, evoked by an intradermal injection of serotonin, it is suggested that mu-opioid receptor in the brain, rather than the spinal dorsal horn, is involved in itch.[17] Thus, the present study was conducted to determine whether cutaneous itch involves mu-opioid receptors in either of the spinal dorsal horn or lower brainstem or in both regions.

Serotonin is a potent pruritogen in mice.[18] Scratching behavior and increase in cutaneous nerve firing following an intradermal injection of serotonin are similar to each other in dose–response relationship and time-course, suggesting the peripheral pruritogenic action of serotonin.[18] An intradermal injection of serotonin causes scratching through 5-HT_2 serotonin receptor in the skin.[10] An injection of serotonin into the hind paw elicits biting and licking of the treated paw, and an injection of dilute formalin licking alone.[5] Systemic injections of the opioid antagonist naloxone and the 5-$HT_{1/2}$ serotonin receptor antagonist methysergide suppress biting, but not licking, induced by serotonin.[5] These findings suggest that serotonin-induced biting behavior is an itch-related response. Thus, in the present experiments, we compared the effects of intrathecal injection *via* a lumbar puncture and intracisternal injection of naloxone on serotonin-induced biting of the hind paw. We also tested whether intracisternal injection of naloxone would affect serotonin-induced scratching of the rostral back, another itch-related response.

MATERIALS AND METHODS

Animals Male ICR mice (Japan SLC, Shizuoka) of 5—7 weeks of age were used. They were housed six per cage under controlled temperature (22±1 °C) and humidity (55±10%). The room was lighted from 7:00 a.m. to 7:00 p.m. Food and water were freely available. The study was approved by the Committee for Animal Experiments at University of Toyama.

Materials Serotonin hydrochloride (Sigma, St. Louis, MO, U.S.A.) and naloxone hydrochloride (Sigma) were dissolved in saline. Serotonin was injected into the malleolus region of the hind paw[5] or the rostral part of the back[19] in a volume of 20 or 50 μl, respectively. Naloxone was injected subcutaneously, intracisternally or intrathecally in a volume of 0.1 ml/10 g weight, 5 μl or 5 μl, respectively. Intracisternal and intrathecal (lumbar puncture) injections were done as described.[20] The weight of naloxone refers to the salt.

Behavioral Observation The animals were placed individually in an acrylic cage for at least 30 min for acclimation. Immediately after serotonin injection, they were put back into the same cage and their behaviors were videotaped with personnel kept out of the observation room. Playing back of the video served for measuring the counts of scratching[19] and the amount of time an animal spent biting.[5]

Data Processing Data are presented as means and

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S.E.M. Statistical significance was analyzed using Dunnett's multiple comparisons or Student's *t*-test; $p<0.05$ was considered significant.

RESULTS

Serotonin-Induced Biting Injections of serotonin (10—300 nmol/site), but not vehicle, into the malleolus region of the hind paw elicited biting of the injected site in a bell-shaped manner with a peak at 100 nmol/site (Fig. 1A). The effect of serotonin (100 nmol/site) peaked during the first 10-min period and almost subsided by 30 min (Fig. 1B). In the following experiments, the cumulative biting responses were counted over a 30-min period after serotonin (100 nmol/site) injection. Subcutaneous injections of naloxone (0.3, 1 mg/kg) produced a dose-dependent inhibition of serotonin-induced biting (Fig. 1C). Intracisternal injection of naloxone (1, 10 nmol/site) also produced a dose-dependent inhibition of serotonin-induced biting, but intrathecal injection of naloxone (10 nmol/site) was without effect (Fig. 1C).

Serotonin-Induced Scratching An injection of serotonin at a dose of 100 nmol/site into the rostral part of the back induced scratching of the injected site; the effect peaked during the first 10-min period and almost subsided by 30 min (Fig. 2A). Since doses higher than 240 nmol/site have been shown to be much less potent than the dose of 100 nmol/site,[10,18] the number of scratch bouts was counted over a 30-min period after serotonin (100 nmol/site) injection. Serotonin-induced scratching was significantly inhibited by naloxone at subcutaneous and intracisternal doses of 1 mg/kg and 10 nmol/site, respectively (Fig. 2B). Sensory signals from the rostral back are conveyed to the cervical and thoracic spinal cord, but it is difficult to inject naloxone intrathecally at the cervical and thoracic levels without injury to the spinal cord. In addition, when intrathecal injection of naloxone through a lumbar puncture does not suppress serotonin-induced scratching, it is difficult to rule out the possibility that enough amount of naloxone does not reach the cervical and thoracic spinal cord. Therefore, we did not test the effect of intrathecal naloxone on serotonin-induced scratching.

DISCUSSION

Subcutaneous administration of naloxone inhibited serotonin-induced biting and scratching. The results are similar to the previous reports in which subcutaneous naloxone (1 mg/kg) inhibited both itch-related behaviors.[5,10]

This is the first report of the inhibition of cutaneous itch by central injection of the opioid antagonist. Intracisternal injection of naloxone suppressed two types of itch-related behaviors evoked by serotonin injection into the hind paw and rostral back, suggesting that endogenous opioids, such as enkephalins or endomorphins, are involved in the facilitative regulation of itch signals in the lower brainstem. In support of this idea, intracisternal injections of morphine and mu-opioid receptor agonist [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin induces itch-related behavior, which is inhibited by systemic administration of naloxone in mice.[15] Intracisternal injec-

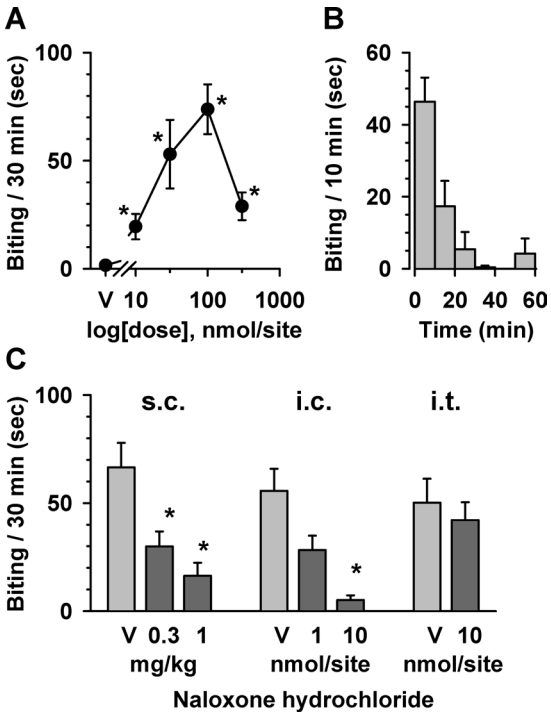

Fig. 1. Inhibition of Serotonin-Induced Biting by Systemic and Intracisternal, But Not Intrathecal, Injections of Naloxone

Serotonin was injected into the malleolus region of the hind paw and the time spent biting the injected paw was recorded. (A) Dose–response relationship of serotonin-induced biting. $n=8$ each group. (B) Time-course of biting following serotonin (100 nmol/site) injection. $n=8$. (C) Effects of naloxone on biting induced by serotonin (100 nmol/site). Naloxone hydrochloride was injected subcutaneously (s.c.), intracisternally (i.c.) or intrathecally (i.t.) 15, 10 or 10 min before serotonin injection, respectively. $n=6$—8. Values are the mean and S.E.M. $*p<0.05$ as compared with the corresponding vehicle (V) control (Dunnett's method).

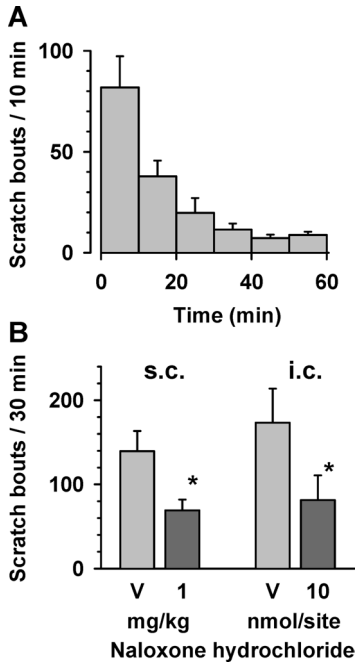

Fig. 2. Inhibition of Serotonin-Induced Scratching by Systemic and Intracisternal Injections of Naloxone

Serotonin (100 nmol/site) was injected intradermally into the caudal back and scratching the injected site was counted. (A) Time-course of scratching following serotonin injection. (B) Effects of naloxone on scratching induced by serotonin. Naloxone hydrochloride was injected subcutaneously (s.c.) or intracisternally (i.c.) 15 or 10 min before serotonin injection, respectively. Values are the mean and S.E.M. for eight animals. $*p<0.05$ as compared with the corresponding vehicle (V) control (Student's *t*-test).

tions of endomorphins, potent endogenous mu-opioid receptor ligands,[21] also induce itch-related behavior.[16] On the other hand, intracisternal injections of delta- and kappa-opioid receptor agonists do not induce itch-related behavior.[15] Thus, naloxone may inhibit cutaneous itch through the action on mu-opioid, but not delta- and kappa-opioid, receptors in the brain.

Serotonin-induced itch-related behavior was not inhibited by intrathecal naloxone. This result is consistent with the findings that an intrathecal injection of morphine does not induce scratching in mice.[15] Epidural and intrathecal injections of opioids at the lumbar level induce itch in human subjects.[22] However, itch is commonly generalized, but not localized to the lower part of the body, and started about 3 h after epidural administration.[23] Morphine progressively spreads in the rostral direction after epidural administration.[23] With these findings taken into account, the present results suggest that mu-opioid receptor in the lower brainstem, but not the spinal cord, is a site of central pruritogenic action of opioids and is involved in the facilitative regulation of itch signaling.

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