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Norbinaltorphimine, a selective κ-opioid receptor antagonist, induces an itch-associated response in mice

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Abstract

We examined the possibility that scratching induced by norbinaltorphimine, a selective κ-opioid receptor antagonist, is due to an itch sensation, using compound 48/80 as control pruritogenic agent. When norbinaltorphimine was injected s.c. into the rostral back, mice scratched the skin around the injection site with their hind paws. Although the intensity of the scratching could not be compared because the dose and injection route were different, the character and time course of the scratching behavior induced by compound 48/80 injected i.d. were similar to those with norbinaltorphimine. The scratching behavior induced by norbinaltorphimine was dose-dependently and significantly inhibited by pretreatment with chlorpheniramine. Compound 48/80-induced scratching was also dose-dependently and significantly inhibited by p.o. pretreatment with chlorpheniramine. The scratching behavior induced by norbinaltorphimine was dose-dependently and significantly inhibited by pretreatment with U-50,488H (*trans*-(±)-2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] acetamide methansulfonate), a κ-opioid receptor agonist. Unexpectedly, the scratching behavior induced by compound 48/80 was also dose-dependently and significantly reduced by pretreatment with U-50,488H. These results suggest that the injection of norbinaltorphimine into the rostral back of the mouse elicited scratching, which may be an itch-associated response. Furthermore, the scratching behavior produced by norbinaltorphimine may be due in part to the release of histamine followed by antagonism of κ-opioid receptors. © 2001 Published by Elsevier Science B.V.

Keywords: Norbinaltorphimine; Itch; Scratching; κ-Opioid receptor; Histamine; Compound 48/80

1. Introduction

Itching is a sensation that provokes a desire to scratch. It is the most common symptom of cutaneous diseases, such as dermatitis, contact dermatitis and urticaria, and accompanies several systemic disorders, such as chronic renal failure and cholestasis. However, its underlying mechanisms are far from being understood.

Recently, Kuraishi and his colleagues reported that s.c. injection of pruritogenic agents into the rostral part of the back of the mouse produced scratching of the injected site with the hind paws, which is an itch-associated behavior (Kuraishi et al., 1995; Andoh et al., 1998). Unexpectedly, we observed that s.c. injection of norbinaltorphimine, a selective and potent κ-opioid receptor antagonist, into the rostral part of the back of the mouse also produced scratching of the injected site with the hind paws. Although algesiogenic agents also produced similar scratching behavior, norbinaltorphimine itself had no significant effect on the nociceptive perception threshold (Endoh et al., 1992; Kamei et al., 1995). On the other hand, it is well recognized that endogenous and exogenous opioidergic mechanisms are involved in processing of the itch sensation (Thomas et al., 1992; Thomas and Hammond, 1995; Tohda et al., 1997). These findings raise the possibility that the scratching induced by norbinaltorphimine might be an itch-associated response. Therefore, in the present study, we examined the possibility that the scratching induced by norbinaltorphimine is due to an itch sensation, and used compound 48/80 as a control pruritogenic agent.

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2. Materials and methods

2.1. Animals

ICR mice (Tokyo Laboratory animals Science, Tokyo, Japan), 6-weeks-old and weighing 26 to 30 g, were used. They had free access to food and water in an animal room that was maintained at 24 ± 1°C with a 12-h light–dark cycle. This study was carried out in accordance with the Declaration of Helsinki and with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Measurement of scratching behavior

Scratching behavior was measured according to the method described by Kuraishi et al. (1995) with some modification. Before the recording of behavior, the mouse was put into an acrylic cage (39 × 26 × 24 cm) for at least 30 min for acclimation. Immediately after subcutaneous or intradermal injection, the mouse was put back into the acrylic cage and its behavior was videotaped for 2 h with the observation room clear of all experimenters. Scratching behavior was counted using the videotape. The mouse generally scratched several times with its hind paws for about 1 s, and a series of these movements was counted as one bout of scratching. Norbinaltorphimine and compound 48/80 were injected s.c. and i.d., respectively, in a volume of 50 μl into the rostral part of the back (around the interscapular level). The hair at the injection site had been shaved off on the day before the injection. U-50,488 (*trans*-(±)-2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] acetamide methansulfonate) or chlorpheniramine was administered p.o. 60 min before the injection of norbinaltorphimine or of compound 48/80. The experiments were blind with respect to the drug treatment and evaluation of scratching behavior.

2.3. Drugs

Compound 48/80, U-50,488H (*trans*-(±)-2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] acetamide methansulfonate) and chlorpheniramine maleate were purchased from Sigma-Aldrich (St. Louis). Norbinaltorphimine was synthesized by Dr. Nagase (Toray Industries). All drugs were dissolved in physiological saline. Citrate buffer at pH 5.5 was used as a vehicle for norbinaltorphimine, since the pH of the saline solution of norbinaltorphimine was 5.5.

2.4. Statistical analysis

Data are expressed as the means with S.E. A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used for the statistical evaluation. A $P < 0.05$ was considered significant.

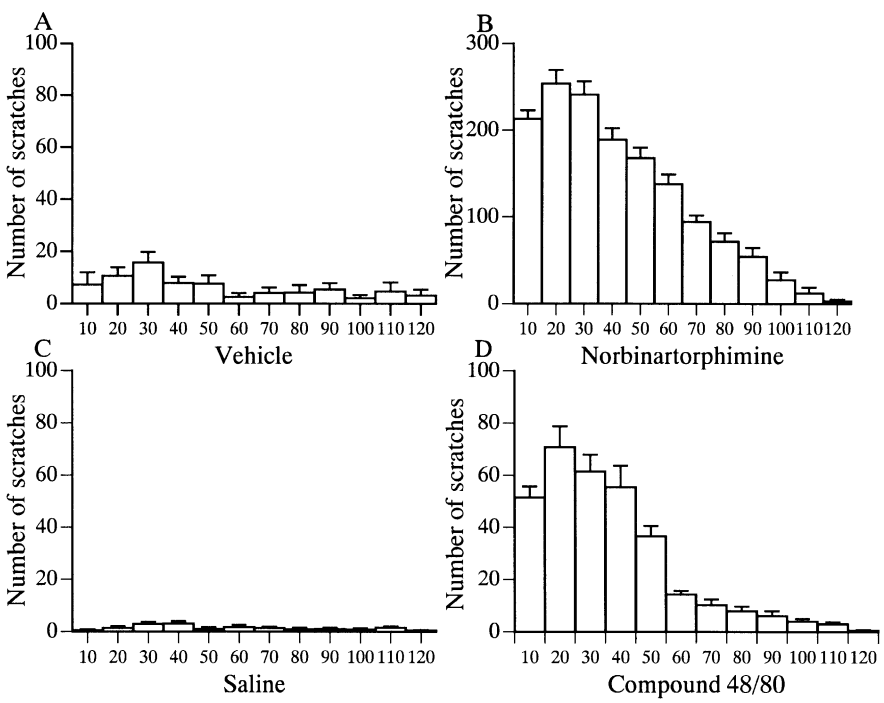

Fig. 1. Time course of scratching after injection of norbinaltorphimine and compound 48/80 in mice. Mice were given an injection of vehicle (citrate buffer at pH 5.5, A), norbinaltorphimine (30 mg/kg, s.c., B), saline (C) or compound 48/80 (100 μg, i.d.). Values represent the means with S.E. for 10 mice in each group.

3. Results

3.1. Norbinaltorphimine-induced scratching behavior

When norbinaltorphimine was injected s.c. into the rostral back, the mice scratched the skin around the injection site with their hind paws. Fig. 1A and B show the time course of scratching behavior for 120 min after the injection of vehicle and norbinaltorphimine (30 mg/kg), respectively. Scratching was first observed within 5 min after the injection of norbinaltorphimine in all cases and then appeared intermittently. The occurrence of behaviors other than scratching, such as grooming and forelimb motions, did not appear to differ between norbinaltorphimine-treated and vehicle-treated mice. Norbinaltorphimine, at doses of 3–30 mg/kg, s.c., produced scratching behavior in a dose-dependent manner, when the number of scratches within 60 min after injection was plotted against the dose (Fig. 2).

Although the intensity of the scratching could not be compared because the dose and injection route were different, the character and time course of scratching behavior induced by compound 48/80 injected i.d. (100 μg, Fig. 1D) (e.g., the first scratching was observed within 5 min after injection, scratching behavior appeared intermittently, scratching behavior lasting more than 60 min) were similar to those with norbinaltorphimine.

3.2. Effects of chlorpheniramine on scratching behavior

Scratching behavior induced by norbinaltorphimine (10 mg/kg, s.c.) was dose-dependently and significantly inhibited by pretreatment with chlorpheniramine at doses of 0.3 to 3 mg/kg, p.o. (Fig. 3A). Compound 48/80 (100 μg, i.d.)-induced scratching was also dose-dependently and significantly inhibited by p.o. pretreatment with chlorpheniramine at doses of 0.3 to 3 mg/kg, p.o. (Fig. 3B).

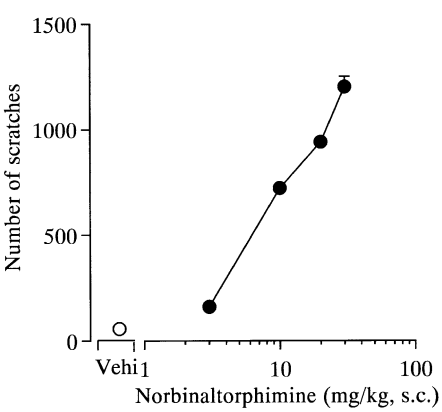

Fig. 2. Dose–response curve for the scratch-inducing effect of norbinartorphimine. Mice were given an s.c. injection of norbinaltorphimine (closed circle) or vehicle (open circle). The number of scratches in 60 min was plotted against the dose. Each point represents the mean with S.E. for 10 mice in each group.

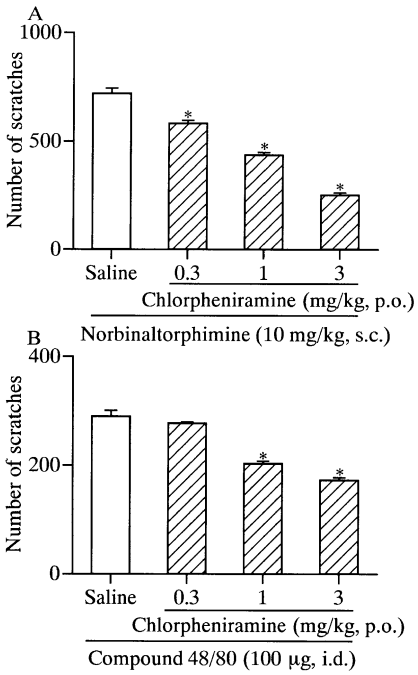

Fig. 3. Effects of chlorpheniramine on norbinaltorphimine (A)- and compound 48/80 (B)-induced scratching. Chlorpheniramine was administered p.o. 60 min before the injection of norbinaltorphimine (10 mg/kg, s.c.) or compound 48/80 (100 μg, i.d.). The number of scratches in 60 min was plotted against the dose. Each column represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ compared with saline-pretreated (open column) group.

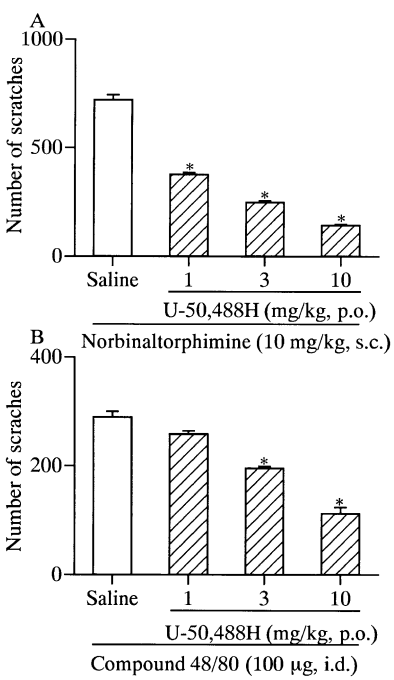

Fig. 4. Effect of U-50,488 on norbinaltorphimine (A)- and compound 48/80 (B)-induced scratching. U-50,488 was administered p.o. 60 min before the injection of norbinaltorphimine (10 mg/kg, s.c.) or compound 48/80 (100 μg, i.d.). The number of scratches in 60 min was plotted against the dose. Each column represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ compared with the saline-pretreated (open column) group.

3.3. Effects of U-50,488H on scratching behavior

To determine whether κ-opioid receptor-mediated mechanisms may be involved in the norbinaltorphimine-induced scratching, the influence of U-50,488H, a κ-opioid receptor agonist, was examined. Scratching behavior induced by norbinaltorphimine (10 mg/kg, s.c.) was dose-dependently and significantly inhibited by pretreatment with U-50,488H at doses of 1 to 10 mg/kg, p.o. (Fig. 4A). Unexpectedly, scratching behavior induced by compound 48/80 was also dose-dependently and significantly reduced by pretreatment with U-50,488H (Fig. 4B).

4. Discussion

The main finding in the present study was that the κ-opioid receptor antagonist, norbinaltorphimine, when injected s.c. into the rostral part of the back in mice, elicited scratching of the injected site with the hind paw. The character and time course of the scratching behavior induced by norbinaltorphimine (e.g., scratching was first observed within 5 min after injection, then appeared intermittently, and lasted for more than 60 min) were similar to those of the scratching behaviors induced by compound 48/80. Subcutaneous injection of norbinaltorphimine into the rostral back elicited scratching of the injection site but not of the face or other areas. Similarly, in compound 48/80-induced scratching, norbinaltorphimine-induced scratching was also suppressed by chlorpheniramine. Thus, it seems likely that the scratching behavior produced by norbinaltorphimine may be due to local skin stimulation and may be mediated, at least in part, by histamine. These similarities between norbinaltorphimine-induced scratching and compound 48/80-induced scratching strongly support the possibility that norbinaltorphimine-induced scratching is due to itch stimulation in the treated skin.

In the present study, we also demonstrated that the scratching behavior produced by norbinaltorphimine was reduced by U-50,488H, a selective κ-opioid receptor agonist. This result suggests the possibility that κ-opioid receptor-mediated mechanisms are involved in the norbinaltorphimine-induced scratching behavior. Interestingly, the scratching induced by compound 48/80 was also suppressed by U-50,488H. Intracranial injection of opioids, especially μ-opioid receptor agonists, induces naloxone-reversible scratching in animals (Thomas et al., 1992; Thomas and Hammond, 1995; Tohda et al., 1997). In mice, intracisternal, but not intradermal, injection of morphine produces dose-dependent facial scratching (Tohda et al., 1997). Recently, Kuraishi et al. (2000) reported that intracisternal injection of δ- and κ-opioid receptor agonists did not elicit scratching of the face or trunk. These results suggest that μ-opioid receptors, but not δ- and κ-opioid receptors, are involved in central processing of the itch sensation. Furthermore, Kuraishi and his coworkers also reported that cutaneous administration of compound 48/80 elicited scratching with the hind paws in mice (Kuraishi et al. 1995; Andoh et al., 1998). With regard to the mechanism of scratching induced by compound 48/80, Andoh et al. (1998) proposed that mast cells were not essential to the mechanism of such scratching since compound 48/80 elicited scratching even in mast cell-deficient (WBB6F1 W/W^v) mice and the frequency of scratching was similar to that in the control $+/+$ mouse. However, compound 48/80 produces the degranulation of mast cells (Ebertz et al., 1987; Lowman et al., 1988) and the release of mediators which produce scratching behaviors. Histamine is released from mast cells and has been thought to be an important mediator of itching. In the present study, we observed that scratching behavior induced by compound 48/80 was inhibited by a histamine H_1 receptor antagonist, chlorpheniramine. Thus, it seems likely that scratching behavior produced by compound 48/80 may be mediated, at least in part, by histamine released from mast cells. Recently, Andrew and Craig (2001) identified histamine-sensitive itch-specific neurons in the dorsal horn of the spinal cord. In this regard, Schmelz (2001) proposed that the activation of these histamine-sensitive itch-specific neurons may account for the pruritic effect of μ-opioid receptor agonists such as morphine and fentanyl. On the other hand, it has been reported that activation of κ-opioid receptors antagonizes various μ-opioid receptor-mediated actions in the brain, such as tolerance, reward and memory processes (Pan, 1998). Thus, it is possible that U-50,488H decreases the activity of histamine-sensitive itch-specific neurons in the dorsal horn of the spinal cord and thereby restores their ability to inhibit itch. These findings taken together suggest that κ-opioid receptor agonists could exert an antipruritic effect.

In conclusion, s.c. injection of the κ-opioid receptor antagonist, norbinaltorphimine, into the rostral back of the mouse elicited scratching, which may be an itch-associated response. Furthermore, the scratching behavior produced by norbinaltorphimine may be due in part to the release of histamine followed by antagonism of κ-opioid receptors. These results provide support for the development of κ-opioid receptor agonists for use as antipruritic drugs.

References

Andoh, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1998. Substance P induction of itch-associated response mediated by cutaneous NK_1 tachykinin receptors in mice. J. Pharmacol. Exp. Ther. 286, 1140–1145.

Andrew, D., Craig, A.D., 2001. Spinothalamic lamina I neurons selectively sensitive to histamine: a central neuronal pathway for itch. Nat. Neurosci. 4, 72–77.

Ebertz, J.M., Hirshman, C.A., Kettelkamp, N.S., Uno, H., Hanifin, J.M., 1987. Substance P-induced histamine release in human cutaneous mast cells. J. Invest. Dermatol. 88, 682–685.

Endoh, T., Matsuura, H., Tanaka, C., Nagase, H., 1992. Nor-binaltorphimine: a potent and selective κ-opioid receptor antagonist with long-lasting activity in vivo. Arch. Int. Pharmacodyn. 316, 30–42.

Kamei, J., Saitoh, A., Ohsawa, M., Suzuki, T., Misawa, M., Nagase, H., Kasuya, Y., 1995. Antinociceptive effects of the selective non-peptidic δ-opioid receptor agonist TAN-67 in diabetic mice. Eur. J. Pharmacol. 276, 131–135.

Kuraishi, Y., Nagasawa, T., Hayashi, K., Satoh, M., 1995. Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. Eur. J. Pharmacol. 275, 229–233.

Kuraishi, Y., Yamaguchi, T., Miyamoto, T., 2000. Itch–scratch response induced by opioids through central mu opioid receptors in mice. J. Biomed. Sci. 7, 248–252.

Lowman, M.A., Benyon, R.C., Church, M.K., 1988. Characterization of neuropeptide-induced histamine release from human dispersed skin mast cells. Br. J. Pharmacol. 95, 121–130.

Pan, Z.Z., 1998. μ-Opposing actions of the κ-opioid receptor. Trends Pharmacol. Sci. 19, 94–98.

Schmelz, M., 2001. A neuronal pathway for itch. Nat. Neurosci. 4, 9–10.

Thomas, D.A., Hammond, D.L., 1995. Microinjection of morphine into the rat medullary dorsal horn produces a dose-dependent increase in facial scratching. Brain Res. 695, 267–270.

Thomas, D.A., Williams, G.M., Iwata, K., Kenshalo Jr., D.R., Dubner, R., 1992. Effects of central administration of opioids on facial scratching in monkeys. Brain Res. 585, 315–317.

Tohda, C., Yamaguchi, T., Kuraishi, Y., 1997. Intracisternal injection of opioids induces itch-associated response through μ-opioid receptors in mice. Jpn. J. Pharmacol. 74, 77–82.