# Intradermal Nociceptin Elicits Itch-Associated Responses Through Leukotriene $B_4$ in Mice

Tsugunoubu Andoh,* Yuichi Yageta,* Hiroshi Takeshima,† and Yasushi Kuraishi*‡
*Department of Applied Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Sugitani, Toyama, Japan; †Department of Biochemistry, Tohoku University Graduate School of Medicine, Seiryo-machi, Aoba-ku, Miyagi, Japan; ‡21st Century COE Program, Toyama Medical and Pharmaceutical University, Sugitani, Toyama, Japan

**Nociceptin, the endogenous peptide ligand for opioid receptor like-1 (ORL1) receptor, has been implicated in the inflammation and pain in the skin. We examined whether nociceptin is a pruritogen in mice. Intradermal injections of nociceptin (1–100 nmol per site) concentration dependently increased scratching in ICR mice; the effect started within 1 min, peaked at 10–20 min, and almost subsided by 30 min. The nociceptin action was absent in ORL1 receptor-deficient (ORL1$^{-/-}$) mice. Systemic, but not local, treatment with naloxone significantly inhibited scratching induced by nociceptin. The action of nociceptin was inhibited by the leukotriene $B_4$ receptor antagonist ONO-4057 and azelastine, which inhibits the action and production of leukotriene $B_4$ in the skin. Prepronociceptin and ORL1 receptor mRNAs were substantially expressed in the skin, whereas their expression levels were very low in the dorsal root ganglia. In the skin, nociceptin- and ORL1 receptor-like immunoreactivities were localized in the epidermis. Administration of nociceptin to primary cultures of keratinocytes from ICR and C57BL/6 (ORL1$^{+/+}$) mice, but not ORL1$^{-/-}$ mice, produced leukotriene $B_4$. The results suggest that nociceptin acts on ORL1 receptor on the keratinocytes to produce leukotriene $B_4$, which induces itch-associated responses in mice.**

Key words: itch/keratinocyte/leukotriene $B_4$/nociceptin/ORL1 receptor/scratching
J Invest Dermatol 123:196–201, 2004

Nociceptin is a peptide with a high affinity for opioid receptor like-1 (ORL1) receptors, which do not bind any of the other endogenous opioid peptides (Bunzow *et al*, 1994; Mollereau *et al*, 1994). It was first identified as a pronociceptive peptide in the brain (Meunier *et al*, 1995; Reinscheid *et al*, 1995). In the case of skin, an injection of nociceptin increases vascular permeability through the release of histamine from mast cells (Kimura *et al*, 2000). An injection of nociceptin into the hind paw induces withdrawal response through the release of substance P from peripheral nerve endings in mice (Inoue *et al*, 1998). Intradermal injections of histamine and substance P elicit an itch sensation in humans (Hägermark *et al*, 1978; Barnes *et al*, 1986) and itch-associated responses in mice (Kuraishi *et al*, 1995; Andoh and Kuraishi, 1998, 2002a; Maekawa *et al*, 2000). These findings raise the possibility that administration of nociceptin to the skin elicits itch. One aim of this study was to test this possibility.

An intradermal injection of substance P increases leukotriene (LT) $B_4$ in the skin and substance P acts on cultured keratinocytes to produce $LTB_4$ (Andoh *et al*, 2001). An intradermal injection of $LTB_4$ at relatively low doses elicits scratching in mice (Andoh and Kuraishi, 1998). Substance P-induced scratching is inhibited by an $LTB_4$ receptor antagonist (Andoh *et al*, 2001). Azelastine and emedastine suppress substance P-induced scratching, in which the blockade of $LTB_4$ action may be involved (Andoh and Kuraishi, 2000, 2002b). In these experiments, considering these findings, we investigated the involvement of $LTB_4$ in the effect of intradermal nociceptin.

Abbreviations: $LTB_4$, leukotriene $B_4$; ONO-4057, 5-[2-(2-carboxyethyl)-3-{6-(4-methoxyphenyl)-5E-hexenyl} oxyphenoxy]valeric acid; ORL1, opioid receptor like-1

## Results

**Nociceptin-induced scratching** An intradermal injection of nociceptin (30 nmol per site) markedly elicited scratching in ICR mice. The onset of scratching was within 1 min after injection in all mice examined. The effect peaked during the first 10-min period and had almost completely subsided by 30 min (Fig 1*a*). The scratching was concentration dependently increased in the range of 1–100 nmol per site of nociceptin (Fig 1*b*). An intradermal injection of nociceptin (30 nmol per site) also elicited scratching in C57BL/6 mice, but it was without effect in ORL1$^{-/-}$ mice (Fig 2).

**Effects of naloxone on nociceptin-induced scratching** Subcutaneous pre-treatment with naloxone (1 mg per kg) significantly inhibited scratching induced by nociceptin (30 nmol per site) (Fig 3*a*). On the other hand, an intradermal injection of naloxone (100 nmol per site) together with nociceptin (30 nmol per site) did not affect nociceptin-induced scratching (Fig 3*b*).

**Effects of anti-allergic agents on nociceptin-induced scratching** Scratching induced by an intradermal injection

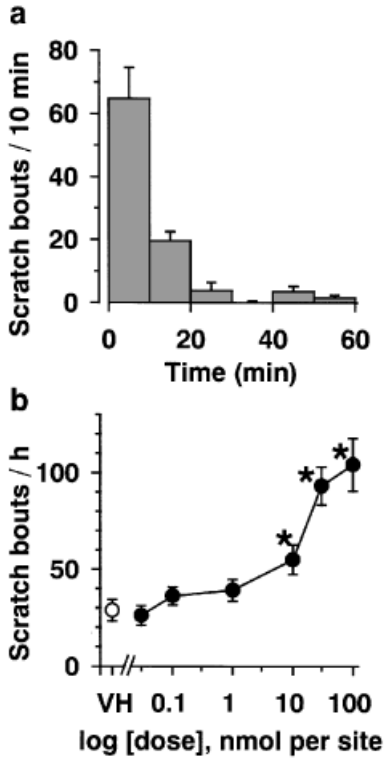


**Figure 1**
**Scratching after intradermal injection of nociceptin in ICR mice.** (*a*) Time-course of scratching after nociceptin (30 nmol per site) injection. (*b*) Dose–response curves for the scratch-inducing effect of nociceptin. Mice were given an intradermal injection of nociceptin or vehicle (VH: saline). Values represent the means ± SEM for eight animals. *$p < 0.05$ when compared with VH (Dunnett's multiple comparisons).

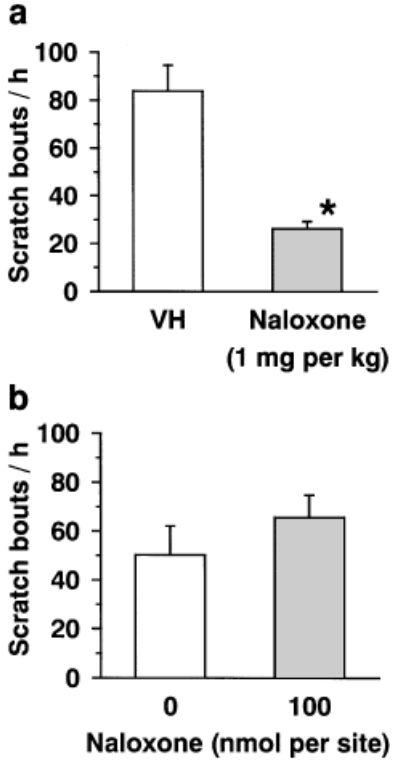


**Figure 3**
**Effect of naloxone on nociceptin-induced scratching in ICR mice.** The mice were given an intradermal injection of nociceptin (30 nmol per site) and scratch bouts were counted for 1 h. (*a*) Naloxone (1 mg per kg) and vehicle (VH: saline) were injected subcutaneously 15 min before nociception. (*b*) Naloxone (100 nmol per site) was injected intradermally together with nociceptin (30 nmol per site). Values represent the means ± SEM for eight animals. *$p < 0.05$ (Student's *t* test).

of nociceptin (30 nmol per site) was significantly inhibited by oral pre-treatment with azelastine (10 mg per kg), but not with terfenadine (30 mg per kg) (Fig 4*a*). The $LTB_4$ receptor antagonist ONO-4057 (30 and100 mg per kg) concentration dependently inhibited the nociceptin-induced scratching (Fig 4*b*).

**Prepronociceptin and ORL1 receptor mRNAs in the dorsal root ganglia and skin** Prepronociceptin mRNA was substantially expressed in the skin, but, on the other hand, the expression level was very low in the dorsal root ganglia in ICR mice (Fig 5*a*). ORL1 receptor mRNA was also substantially expressed in the skin, whereas the expression level was very low in the dorsal root ganglia (Fig 5*b*).

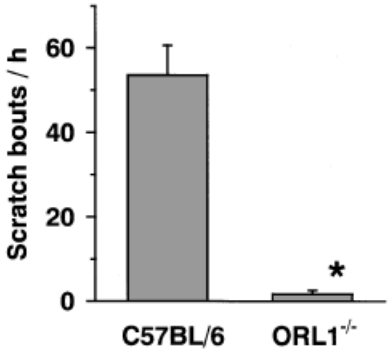


**Figure 2**
**ORL1 receptor deficiency abolishes scratching induced by intradermal injection of nociceptin in mice.** Nociceptin (30 nmol per site) was injected intradermally into C57BL/6 ($ORL1^{+/+}$) and $ORL1^{-/-}$ mice. Values represent the means ± SEM for eight animals. *$p < 0.05$ (Student's *t* test).

**Distribution of nociceptin and ORL1 receptor in the skin** ORL1 receptor-like immunoreactivity was localized in the epidermis, especially its superficial layers, of the back skin and absent there in $ORL1^{-/-}$ mouse (Fig 6, *upper panels*). Nociceptin-like immunoreactivity was also localized in the superficial layer of epidermis, which was not affected by the deficiency of ORL1 receptors (Fig 6, *lower panels*). Similar results were obtained in other four C57BL/6 mice and four $ORL1^{-/-}$ mice.

**Production of $LTB_4$ in keratinocytes** In the above experiments, ORL1 receptor-like immunoreactivity was localized in the epidermis, the major cell of which is keratinocytes. Since $LTB_4$ is produced by keratinocytes (Andoh *et al*, 2001) and elicits scratching at relatively low intradermal doses (Andoh and Kuraishi, 1998), we examined whether nociceptin would increase the production of $LTB_4$ in the keratinocytes *in vitro*. A bath application of nociceptin (10 and 100 μM) increased the production of $LTB_4$ in a concentration-dependent manner in primary cultures of keratinocytes from ICR and C57BL/6 mice (Fig 7). The effect of nociceptin (100 μM) was abolished by zileuton (10 μM) and deficiency in ORL1 receptors (Fig 7).

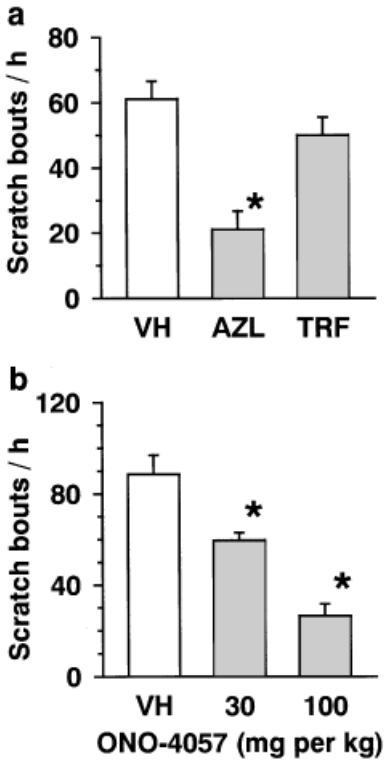


**Figure 4**
**Effects of anti-allergic agents on nociceptin-induced scratching in ICR mice.** The mice were given an intradermal injection of nociceptin (30 nmol per site) and scratch bouts were counted for 1 h. (*a*) Azelastine (AZL; 10 mg per kg) and terfenadine (TRF; 30 mg per kg) were administered orally 1 h before nociceptin. (*b*) ONO-4057 (30–100 mg per kg) was administered orally 1 h before nociceptin. Values represent the means ± SEM for eight animals. *$p < 0.05$ when compared with vehicle (VH) (Dunnett's multiple comparisons).

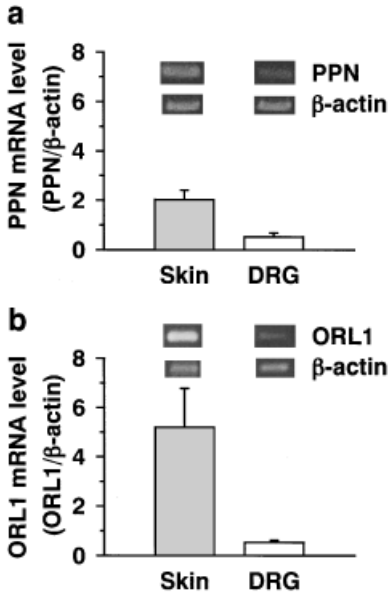


**Figure 5**
**Expression of prepronociceptin (PPN) and ORL1 receptor mRNAs in the mouse skin and dorsal root ganglia (DRG).** The expression levels of mRNAs of prepronociceptin and ORL1 receptor were determined with RT-PCR method (for detailed procedures see Materials and Methods). The expression level was normalized to the level of β-actin mRNA. Typical examples of the bands of PPN and β-actin mRNAs are shown above the corresponding columns. Values represent the means ± SEM for three animals.

## Discussion

**Nociceptin-induced scratching** One aim of this study was to test whether nociceptin would elicit itch. An intradermal injection of nociceptin elicited scratching in mice. The effective dose range (1–100 nmol per site) of nociceptin was similar to those of substance P (Andoh *et al*, 1998), histamine (Maekawa *et al*, 2000), and serotonin (Yamaguchi *et al*, 1999). Systemic administration of the opioid antagonist naloxone markedly inhibited nociceptin-induced scratching, whereas a local injection of naloxone was without effect. Scratching induced by substance P and serotonin in healthy mice and the spontaneous scratching of mice with chronic dermatitis or xerosis are inhibited by systemic injections of opioid antagonists (Andoh *et al*, 1998; Yamaguchi *et al*, 1999, 2001; Miyamoto *et al*, 2002). In mice with chronic dermatitis, systemic administration of the opioid antagonist naltrexone suppresses the spontaneous scratching without effect on the increased firing of cutaneous branch of sensory nerve (Maekawa *et al*, 2002). Thus, the results suggest that opioid receptors in the skin (for example, on the peripheral terminals of sensory neurons) are not involved in the nociceptin action and that naloxone inhibits nociceptin-induces scratching through central action. This idea is consistent with the findings that nociceptin does not show any activity at the opioid receptors (Mahis *et al*, 1997). Opioid antagonists reduce experimentally elicited itch (Bernstein *et al*, 1982) and itching of patients with pruritic diseases such as cholestasis, chronic urticaria, and atopic dermatitis (Monroe 1989; Bergasa *et al*, 1995). Opioids may induce pruritus through mu-opioid receptors in the central nervous system (Szarvas *et al*, 2003). Thus, the inhibition of nociceptin-induced scratching by systemic naloxone suggests that the scratching is an itch-associated response.

In these experiments, an injection of nociceptin into the rostral back elicited scratching. On the other hand, an injection of nociceptin into the hind paw was shown to elicit withdrawal response in mice (Inoue *et al*, 1998). In their experiments, mice were suspended and could not lick and bite the hind paw, although painful and pruritic stimulation of the hind paw induces licking (or flinching) and biting behaviors, respectively, in mice (Hagiwara *et al*, 1999). Thus, it is possible that the withdrawal-like response of suspended animal is related to itch. It is also possible that nociceptin is more effective in exciting nociceptors in the plantar skin than in the back skin, and a subcutaneous injection of nociceptin into the plantar skin elicits pain in mice. To determine whether intraplantar nociceptin elicits itch and/or pain, this peptide should be injected into the plantar region of freely moving animals.

**Origin and target cells of nociceptin** Nociceptin-induced scratching was abolished by deficiency of ORL1 receptors, suggesting that the action is mediated by ORL1 receptors. It is suggested that keratinocytes are the main origin cells for nociceptin in the skin. The expression level of prepronociceptin mRNA was relatively high in the skin and nociceptin-

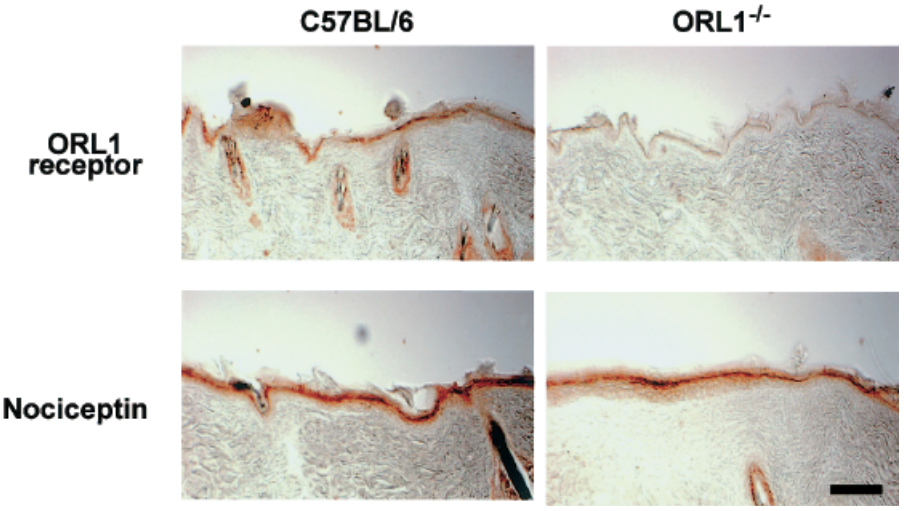


**Figure 6**
**Typical examples of distribution of nociceptin and ORL1 receptor in the mouse skin.** Nociceptin and ORL1 receptor were immunostained in the back skin of C57BL/6 (ORL1$^{+/+}$) and ORL1$^{-/-}$ mice. *Scale bar* = 50 μm.

like immunoreactivity was localized in the superficial layers of the epidermis. These results strongly suggest that keratinocytes in the superficial layers synthesize and contain nociceptin. On the other hand, there were no nociceptin-immunoreactive nerve fibers in the skin. The expression level of prepronociceptin mRNA was low in the dorsal root ganglia. The result is consistent with the finding that the level of prepronociceptin mRNA is very low in the dorsal root ganglia of normal rats (Andoh *et al*, 1997).

These results strongly suggest that keratinocytes are the main target cells for intradermal nociceptin, and co-localization of nociceptin and its receptor in the keratinocytes suggest the autocrine-like action of nociceptin. The expression level of ORL1 receptor mRNA was relatively high in the skin and ORL1 receptor-like immunoreactivity was localized in the superficial layers of the epidermis. The immunoreactivity was absent in the skin of ORL1$^{-/-}$ mice. On the other hand, the expression level of ORL1 receptor mRNA was very low in the dorsal root ganglia and there were no nerve fibers immunoreactive for ORL1 receptors. But we do not rule out the possibility of nociceptin action on primary afferents. The splice variants of ORL1 receptor were reported to be present in the dorsal root ganglia (Xie *et al*, 1999), and nociceptin was suggested to act on primary afferents (Inoue *et al*, 1998).

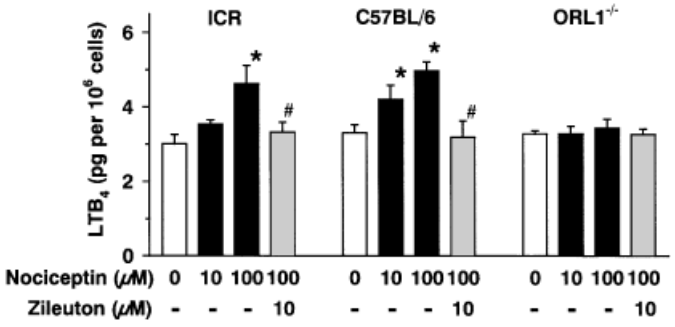


**Figure 7**
**Nociceptin-induced production of $LTB_4$ in mouse keratinocytes.** Keratinocytes were prepared from the epidermis of the back skin of ICR, C57BL/6, and ORL1$^{-/-}$ mice. Nociceptin (10 and 100 μM) was administered to primary cultures of the mouse keratinocytes, and 5 min later the supernatant was taken for the assay. Zileuton (10 μM) was administered 1 h before nociceptin. Values represent the means ± SEM for six animals, * and $^{\#}p<0.05$ when compared with the group without nociception and nociceptin (100 μM) alone (Dunnett's multiple comparisons).

**$LTB_4$ involvement in nociceptin-induced scratching** Nociceptin-induced scratching was inhibited by the $LTB_4$ antagonist ONO-4057, which inhibits $LTB_4$-induced scratching in mice (Andoh *et al*, 2001). An intradermal injection of $LTB_4$ at relatively low doses elicits scratching in mice (Andoh and Kuraishi, 1998). Therefore, $LTB_4$ may play an important role in the nociceptin-induced scratching. Azelastine (10 mg per kg) suppressed nociceptin-induced scratching. This dosage inhibits $LTB_4$ production in the skin and $LTB_4$-induced scratching in mice (Andoh and Kuraishi, 2002b). In addition, azelastine suppresses depolarization-induced increase in intracellular $Ca^{2+}$ concentration in cultured dorsal root ganglion neurons (Ohtsuka *et al*, 2003). These actions may be involved in the inhibition by azelastine of nociceptin-induced scratching.

Nociceptin induced the production of $LTB_4$ in primary cultures of keratinocytes. This effect was abolished by the deficiency of ORL1 receptors, suggesting the involvement of ORL1 receptors. The mechanism of the nociceptin-induced production of $LTB_4$ is not clear; however, ORL1 receptor is a pertussis toxin-sensitive-G-protein (Gi)-coupled receptor (Hawes *et al*, 1998) and Gi activates phospholipase C (Ueda *et al*, 1995). Activated phospholipase C increases intracellular $Ca^{2+}$ concentration. In our preliminary experiments, nociceptin increased intracellular $Ca^{2+}$ concentration in mouse keratinocytes (Andoh and Kuraishi: unpublished observation). An increase in intracellular $Ca^{2+}$ activates the secretory- and cytosolic-type phospholipase $A_2$ (Clark *et al*, 1990). Such processes may lead to the production of $LTB_4$ in keratinocytes.

**Role of histamine in nociceptin-induced scratching** Nociceptin-induced scratching was not affected by the $H_1$ histamine receptor antagonist terfenadine. Since the same dosage inhibits histamine-induced scratching in mice (Ohtsuka *et al*, 2001), histamine may not play an important role in nociceptin-induced scratching. An intradermal injection of nociceptin increases vascular permeability through the release of histamine from mast cells in rats

(Kimura *et al*, 2000). But terfenadine suppresses plasma extravasation, but not scratching, induced by immediate allergy (Ohtsuka *et al*, 2001), suggesting that mediator(s) involved in scratching is different from those of plasma extravasation.

**Summary** Nociceptin released from epidermal keratinocytes may stimulate ORL1 receptors on the keratinocytes through autocrine mechanisms. Stimulated keratinocytes produce $LTB_4$, which acts on primary afferents to induce itch sensation. In our preliminary experiments, nociceptin was increased in the inflamed skin of itching mice (NC strain), a mouse model of atopic dermatitis (Yamaguchi *et al*, 2001). Although further experiments are needed, it is possible that nociceptin is involved in inflammatory itchy skin diseases. Nociceptin and ORL1 receptor in the skin may be possible target molecules for new antipruritic agents.

## Materials and Methods

**Animals** We used male ICR mice (4–5 weeks old; Japan SLC, Shizuoka, Japan), male C57BL/6 (ORL1$^{+/+}$; Japan SLC), and ORL1 receptor-deficient mice (ORL1$^{-/-}$) with C57BL/6 genetic background (9–10 weeks old). They were housed 7–8 per cage in a room under controlled temperature (22 $\pm$ 1°C), humidity (55 $\pm$ 10%), and light (lights on from 07:00 to 19:00 h). Food and water were freely available. Procedures in the animal experiments were approved by the Committee for Animal Experiments at Toyama Medical and Pharmaceutical University.

**Materials** Nociceptin (Peptide Institute, Minoh, Japan) was dissolved in physiological saline. Azelastine (Eizai, Tokyo, Japan), 5-[2-(2-carboxyethyl)-3-oxyphenoxy] valeric acid (ONO-4057; Ono Pharmaceutical, Osaka, Japan), and terfenadine (Sigma Chemical, St Louis, Missouri) were suspended in 0.5% (wt/vol) sodium carboxymethyl cellulose (Wako Pure Chemical, Osaka, Japan). In *in vivo* experiments, azelastine and terfenadine were administered orally 30 min before nociceptin injection, and ONO-4057 before 1 h. Naloxone hydrochloride (Sigma Chemical) was dissolved in physiological saline and injected subcutaneously 15 min before nociceptin or intradermally together with nociceptin. Zileuton (Ono Pharmaceutical) was dissolved in dimethyl sulfoxide.

**Behavioral experiments** The hair was clipped over the rostral part of the mouse back. The next day, nociceptin was injected intradermally in a volume of 50 μL into the interscapular region using a 27-gauge needle connected to a microsyringe via PE-10 tubing. Before behavior observation, the mice (four animals per observation) were put into an acrylic cage composed of four cells (13 $\times$ 9 $\times$ 30 cm) for at least 1 h for acclimation. Immediately after intradermal injection, the animals were put back into the same cells and their behaviors were videotaped using an 8 mm video camera for 1 h with personnel kept out of the observation room. Playing back of the video served for counting scratching behavior. The mice stretched either hind paw toward the injection site, leaned the head toward the hind paw, and rapidly scratched several times for about 1 s. A series of these movements was counted as one bout of scratching (Kuraishi *et al*, 1995); scratch bout was considered to end when the mouse lowered its hind paw.

**RT-PCR** The dorsal root ganglia (C1–T13 levels) and skin were removed, immediately frozen in liquid nitrogen, and stored at −80°C until assay. Total RNA was extracted with RNAzol B (Cinna/Biotecx, Houston, Texas) and treated with DNase I (Takara, Shiga, Japan). Total RNA (1 μg) was incubated at 37°C for 60 min with a mixture of 100 U of reverse transcriptase (Superscript II, Gibco BRL, Rockville, Maryland), 1 $\times$ buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 20 mM dithiothreitol), 0.5 mM dNTP, and 50 pmol oligo$(dT)_{16}$ primer. Thereafter, the reaction mixture was incubated at 99°C for 5 min and then chilled on ice for 5 min. An aliquot (1 μL) of the RT product was mixed with PCR solution containing 1.5 mM $MgCl_2$, 1 $\times$ buffer, 1.5 U *Taq* DNA polymerase (Applied Biosystems, Branchburg, New Jersey), and 50 pmol of sense and antisense primers pair. The sequences of primers were as follows: prepronociceptin (sense), 5′-gtgactctgagcagctcagc-3′; prepronociceptin (antisense), 5′-ttctggttggccaacttccg-3′; ORL1 (sense) 5′-gatcgagtgcctggtggagatcc-3′; ORL1 (antisense) 5′-catgggcaggtccacgcctagtc-3′; β-actin (sense), 5′-tcagaaggactcctatgtgg-3′; and β-actin (antisense), 5′-tctctttgatgtcacgcacg-3′. A program for prepronociceptin was 33 cycles (94°C for 1 min, 70°C for 1 min, 72°C for 1 min), that for ORL1 33 cycles (94°C for 1 min, 55°C for 1 min, 72°C for 1 min), and that for β-actin 28 cycles (94°C for 1 min, 55°C for 1 min, 72°C for 1 min). After the separation of PCR product with gel electrophoresis and ethidium bromide staining, the density of the band of predicted size was analyzed using NIH Image software (National Institute of Health, Bethesda, Maryland).

**Immunohistochemistry** The animals were anesthetized with diethyl ether and euthanized via cervical dislocation. After exsanguination, the skin was removed from the rostral back, frozen in liquid nitrogen, and cut into sections of 20 μm thickness. The sections were fixed by incubation with acetone at 4°C for 5 min and washed 4 times for 5 min with phosphate-buffered saline (pH 7.4). For quenching of endogenous peroxidase, the samples were treated with 0.3% $H_2O_2$ in methanol for 20 min, washed 4 times with phosphate-buffered saline, and then treated with the blocking buffer (0.5% fetal bovine serum in phosphate-buffered saline containing 0.1% Tween 20) for 30 min. They were then incubated with goat anti-nociceptin or goat anti-ORL1 receptor antibody (diluted 1:500 with blocking buffer; Santa Cruz Biotechnology, Santa Cruz, California) at 4°C overnight. After being washed with phosphate-buffered saline, they were incubated with horseradish peroxidase-conjugated donkey anti-goat IgG (diluted 1:2000 with blocking buffer; Santa Cruz Biotechnology) at room temperature for 1.5 h. After being washed with phosphate-buffered saline, the section was re-washed four times with 0.05 M Tris-HCl buffer (pH 7.6) containing 0.9% NaCl. Finally, they were treated with 0.2 mg per ml 3,3-diaminobenzidine (Wako Pure Chemical) in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.9% NaCl and 0.05% $H_2O_2$ at room temperature for 30 min. The staining was observed using a light microscope (AX80, Olympus, Osaka, Japan) with a CCD camera (Axio Cam, Carl Zeiss, Jena, Germany).

**Enzyme immunoassay for $LTB_4$** Keratinocytes were prepared from neonatal mice and cultured (Andoh *et al*, 2001). Nociceptin and zileuton (1 h before nociceptin) were added to the medium of primary cultures of mouse keratinocytes ($10^6$ cells per well). Samples were taken from the medium 5 min after nociceptin administration and diluted with enzyme immunoassay buffer (Cayman Chemical, Ann Arbor, Michigan) for the assay. The amount of $LTB_4$ was determined using an enzyme immunoassay kit (Cayman Chemical).

**Data processing** All data are presented as means and SEM. Statistical significance was analyzed using Dunnett's multiple comparisons or Student's *t* test; $p < 0.05$ was considered significant.

DOI: 10.1111/j.0022-202X.2004.22704.x

Manuscript received December 25, 2003; revised February 5, 2004; accepted for publication February 25, 2004

Address correspondence to: Yasushi Kuraishi, Department of Applied Pharmacology, Faculty of Pharmaceutical University, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 9301-0194, Japan. Email: kuraisiy@ms.toyama-mpu.ac.jp

## References


Andoh T, Itoh M, Kuraishi Y: Nociceptin gene expression in rat dorsal root ganglia induced by peripheral inflammation. Neuroreport 8:2793–2796, 1997

Andoh T, Katsube N, Maruyama M, Kuraishi Y: Involvement of leukotriene $B_4$ in substance P-induced itch-associated response in mice. J Invest Dermatol 117:1621–1626, 2001

Andoh T, Kuraishi Y: Intradermal leukotriene $B_4$, but not prostaglandin $E_2$, induces itch-associated responses in mice. Eur J Pharmacol 353:93–96, 1998

Andoh T, Kuraishi Y: Involvement of blockade of leukotriene $B_4$ action in antipruritic effects of emedastine in mice. Eur J Pharmacol 406:149–152, 2000

Andoh T, Kuraishi Y: Nitric oxide enhances substance P-induced itch-associated responses in mice. Br J Pharmacol 138:202–208, 2002a

Andoh T, Kuraishi Y: Inhibitory effects of azelastine on substance P-induced itch-associated response in mice. Eur J Pharmacol 436:235–239, 2002b

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

Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind PW: Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. Br J Pharmacol 88:741–745, 1986

Bergasa NV, Alling DW, Talbot TL, *et al*: Effects of naloxone infusions in patients with the pruritus of cholestasis. A double-blind, randomized, controlled trial. Ann Int Med 123:161–167, 1995

Bernstein JE, Swift RM, Soltani K, Lorincz AL: Antipruritic effect of an opiate antagonist, naloxone hydrochloride. J Invest Dermatol 78:82–83, 1982

Bunzow JR, Saez C, Mortrud M, Bouvier C, Williams JT, Low M, Grandy D: Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a $\mu$, $\delta$ or $\kappa$ opioid receptor subtype. FEBS Lett 347:284–288, 1994

Clark JD, Milona N, Knopf JL: Purification of a 110-kilodalton cytosolic phospholipase A2 from the human monocytic cell line U937. Proc Natl Acad Sci USA 87:7708–7712, 1990

Hägermark O, Hökfelt T, Pernow B: Flare and itch induced by substance P in human skin. J Invest Dermatol 71:233–235, 1978

Hagiwara K, Nojima H, Kuraishi Y: Serotonin-induced biting of the hind paw is itch-related response in mice. Pain Res 14:53–59, 1999

Hawes BE, Fried S, Yao X, Weig B, Graziano MP: Nociceptin (ORL-1) and mu-opioid receptors mediate mitogen-activated protein kinase activation in CHO cells through a Gi-coupled signaling pathway: Evidence for distinct mechanisms of agonist-mediated desensitization. J Neurochem 71:1024–1033, 1998

Inoue M, Kobayashi M, Kozaki S, Zimmer A, Ueda H: Nociceptin/orphanin FQ-induced nociceptive responses through substance P release from peripheral nerve endings in mice. Proc Natl Acad Sci USA 95: 10949–10953, 1998

Kimura T, Kitaichi K, Hiramatsu K, *et al*: Intradermal application of nociceptin increases vascular permeability in rats: The possible involvement of histamine release from mast cells. Eur J Pharmacol 407:327–332, 2000

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

Maekawa T, Nojima H, Kuraishi Y: Itch-associated responses of afferent nerve innervating the murine skin: Different effects of histamine and serotonin in ICR and ddY mice. Jpn J Pharmacol 84:462–466, 2000

Maekawa T, Yamaguchi-Miyamoto T, Nojima H, Kuraishi Y: Effects of naltrexone on spontaneous itch-associated responses in NC mice with chronic dermatitis. Jpn J Pharmacol 90:193–196, 2002

Mathis JP, Ryan-Moro J, Chang A, Hom JS, Scheinberg DA, Pasternak GW: Biochemical evidence for orphanin FQ/nociceptin receptor heterogeneity in mouse brain. Biochem Biophys Res Commun 230:462–465, 1997

Meunier JC, Mollereau C, Toll L, *et al*: Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 377:532–535, 1995

Miyamoto T, Nojima H, Shinkado T, Nakahashi T, Kuraishi Y: Itch-associated response induced by experimental dry skin in mice. Jpn J Pharmacol 88:285–292, 2002

Mollereau C, Parmentier M, Mailleux P, *et al*: ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett 341:33–38, 1994

Monroe EW: Efficacy and safety of nalmefene in patients with severe pruritus caused by chronic urticaria and atopic dermatitis. J Am Acad Dermatol 21:135–136, 1989

Ohtsuka E, Kawai S, Ichikawa T, *et al*: Roles of mast cells and histamine in mosquito bite-induced allergic itch-associated responses in mice. Jpn J Pharmacol 86:97–105, 2001

Ohtsuka E, Kawai S, Nojima H, Andoh T, Kamimura K, Kuraishi Y: Inhibitory effect of azelastine on allergic itch-associated response in mice sensitized with mosquito salivary glands extract. J Pharmacol Sci 91:263–267, 2003

Reinscheid RK, Nothacker HP, Bourson A, *et al*: Orphanin FQ: A neuropeptide that activates an opioidlike G protein-coupled receptor. Science 270: 792–794, 1995

Szarvas S, Harmon D, Murphy D: Neuraxial opioid-induced pruritus: A review. J Clin Anesth 15:234–239, 2003

Ueda H, Miyamae T, Hayashi C, *et al*: Protein kinase C involvement in homologous desensitization of delta-opioid receptor coupled to Gi1-phospholipase C activation in Xenopus oocytes. J Neurosci 15: 7485–7499, 1995

Xie GX, Meuser T, Pietruck C, Sharma M, Palmer PP: Presence of opioid receptor-like (ORL1) receptor mRNA splice variants in peripheral sensory and sympathetic neuronal ganglia. Life Sci 64:2029–2037, 1999

Yamaguchi T, Maekawa T, Nishikawa Y, *et al*: Characterization of itch-associated responses of NC mice with mite-induced chronic dermatitis. J Dermatol Sci 25:20–28, 2001

Yamaguchi T, Nagasawa T, Satoh M, Kuraishi Y: Itch-associated response induced by intradermal serotonin through 5-$HT_2$ receptors in mice. Neurosci Res 35:77–83, 1999