

Involvement of central μ -opioid system in the scratching behavior in mice, and the suppression of it by the activation of κ -opioid system

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Received 27 June 2003; received in revised form 28 July 2003; accepted 5 August 2003

Abstract

The role of central μ - and κ -opioid receptors in the regulation of itch sensation was examined using pruritogen-induced mouse scratching behavior model. Intracerebroventricular administration of β -funaltrexamine, a selective μ -opioid receptor antagonist, inhibited the scratching behavior induced by intradermal substance P, but subcutaneous administration of β -funaltrexamine did not. Similarly, the scratching inhibitory activity of subcutaneously administered TRK-820, (–)-17-(cyclopropylmethyl)-3, 14 β -dihydroxy-4, 5 α -epoxy-6 β -[N-methyl-trans-3-(3-furyl) acrylamido] morphinan hydrochloride, a κ -opioid receptor agonist, was antagonized by intracerebroventricular administration of nor-binaltorphimine (10 μ g/site), a κ -opioid receptor antagonist, but was not by subcutaneous administration of nor-binaltorphimine. In addition, the scratching induced by the direct activation of central μ -opioid receptor by intracisternal morphine was significantly and dose-dependently inhibited by subcutaneous administration of TRK-820. Taken all together, it is suggested that the central μ -opioid receptors play a role in the processing of itch sensation, and the activation of central κ -opioid receptors antagonize the central μ -opioid receptor mediated itch processing, thereby suppressing itch sensation.

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Keywords: Pruritus; Opioid system; TRK-820; CNS (central nervous system); Behavior; (Mouse)

1. Introduction

Pruritus is defined as a sensation that provokes urge to scratch, and may be one of the most common symptoms associated with dermatitis (Hanifin, 1986) and some internal diseases (Gilchrest, 1982). Antihistamines and their ameliorated drugs have been used to treat pruritus in most case of urticaria (Kennard and Ellis, 1991) and mastocytosis (Friedman et al., 1993). Their modes of actions are based on the antagonism against histamine H_1 receptor and the inhibition of histamine release from mast cells (Wahlgren, 1992). On the other hand, antihistamine resistant pruritus is more common in clinical settings and is observed in patients with many symptoms such as atopic dermatitis (Wahlgren, 1992), chronic obstructive liver disease (Duncan et al., 1984) and chronic renal failure (Ostlere et al., 1994). In such a case, it is believed that some endogenous ligands other than histamine play a role in provoking itch sensation.

There are observations indicating the involvement of μ -opioid receptor system in itch processing. For example, naloxone and naltrexone, μ -opioid receptor antagonists show antipruritic effects in patients with chronic cholestasis, chronic renal failure and atopic dermatitis (Monroe, 1989; Peer et al., 1996; Bergasa et al., 1999). Similar effect is observed in animals as μ -opioid receptor antagonist naloxone inhibited the intradermal substance P-induced mouse-scratching behavior (Andoh et al., 1998). Hence, endogenous μ -opioid receptor ligand and receptor are possibly involved in itch processing although their sites of action remain unclear. Furthermore, both systemic and central administration of morphine produce pruritus, of interest, a more frequent incidence of itch occurs with spinal administration (Ballantyne et al., 1988). Similarly, central administration of morphine induced scratching behavior in mouse and monkey (Tohda et al., 1997; Thomas et al., 1993). Those reports raise the possibility that μ -opioid receptor agonist acts as not only mast cell degranulating agent as reported previously (Barke and Hough, 1993) but also as the pruritogen in the central nervous systems (CNS). Since it is

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reported that pruritus arises from the stimulation on itch receptors existing on a free unmyelinated nerve ending in the dermo-epidermal junction (Schmelz et al., 1997) and the generated itch signal enters into the CNS via dorsal root ganglion, ascend the spinothalamic tract, and transmitted to cortex cerebri through thalamus (Fjellner, 1981), μ -opioid receptor system may play a role somewhere in this pathway.

In addition to the μ -opioid receptor system, recently we and others show the involvement of κ -opioid receptor system in the regulation of itch sensation (Kamei and Nagase, 2001) and the itch inhibitory activity of κ -opioid receptor agonists (Cowan and Kehner, 1997; Togashi et al., 2002) using experimental mouse models. In these reports, the possibility that κ -opioid receptor agonists are effective against both the antihistamine-sensitive and antihistamine-resistant pruritus is indicated. Question remains for the antipruritic mechanisms of these κ -opioid receptor agonists.

Our purpose in the present study is to know the relationship between the μ - and κ -opioid system in terms of the modulation of itch sensation. To answer the question, the effect of μ - and κ -opioid receptor agonists and antagonists on the pruritogen-induced mouse-scratching behavior was investigated. Scratching behavior induced by peripheral stimulation by substance P was suppressed by central not by peripheral administration of small amount of β -funaltrexamine. Subcutaneous administration of TRK-820, (–)-17-(cyclopropylmethyl)-3, 14 β -dihydroxy-4, 5 α -epoxy-6 β -[N-methyl-trans-3-(3-furyl) acrylamido] morphinan hydrochloride, a κ -opioid receptor agonist (Nagase et al., 1998), inhibited scratching behavior induced by intradermal injection of substance P, and the effect is neutralized by the central not by peripheral administration of small amount of nor-binaltorphimine, a κ -opioid receptor antagonist. TRK-820 also suppressed the scratching behavior induced by the intracisternal injection of morphine. These results suggest that the activation of central μ -opioid system is involved in the scratching behaviors and the activation of central κ -opioid system antagonizes the action.

2. Material and methods

2.1. Animals

Male ddY mice (Japan SLC, Shizuoka, Japan), 4–5 weeks of age were used. The animals were housed five per cage under a controlled 12-h light and dark cycle and allowed free access to food pellets and tap water. This study was conducted in accordance with the guidelines for Care and Use of Laboratory Animals in Toray Research Laboratories.

2.2. Drugs

Substance P (Peptide Institute, Osaka, Japan) was dissolved in phosphate buffered saline, pH 7.4 (Gibco BRL,

New York, USA). β -Funaltrexamine (synthesized by Toray Industries) was dissolved in distilled water. Morphine hydrochloride (Takeda Chemical Industries, Tokyo, Japan), TRK-820 (synthesized by Toray Industries), ketotifen (Sigma, St. Louis, USA) and nor-binaltorphimine (synthesized by Toray Industries) were all dissolved in physiological saline.

2.3. Observation of the scratching behavior

The scratching behavior was observed according to the method described already (Tohda et al., 1997; Kuraishi et al., 1995). Briefly, 1 day before testing, hair was removed at the injection sites by clippers. On the testing day, mice were individually placed in sections of the observation cage (each cage consisted of four sections of 10 \times 14 \times 30 cm in size) to acclimate for about 30 min. After the acclimation, a test substance and a pruritogen were administered to the mice. Then, the behavior was recorded by an unattended video camera and scratching frequency was determined by replaying the recorded videotapes. Usually, mice scratch by their paws several times a second, therefore, a series of these scratches was counted as one scratch event.

2.3.1. Substance P-induced scratching behavior

Substance P (250 nmol/site) was injected intradermally in a volume of 50 μ l/site into the rostral part of the back with a 27 gauge needle. The test compound, TRK-820 (0.3, 1, 3, 10 μ g/kg, s.c., – 30 min) or β -funaltrexamine (10 μ g/site, i.c.v. or s.c., – 24 h), was administered before the substance P injection. The number of scratches only toward the injection site was counted and that of other sites such as ears and face was discarded. Nor-binaltorphimine (10 μ g/site, i.c.v. or s.c.) was treated 24 h before the TRK-820 injection. Intracerebroventricularly administration of nor-binaltorphimine or β -funaltrexamine was developed by the description (Haley and McCormick, 1957). A microsyringe (Hamilton, 702-04, lure chip, LT standard type) was connected with a needle with a double diameter tip (Natsume Seisakusho, KN-386, special injection needle, 0.4 \times 2 + 0.7 \times 10 mm). The needle was moved at right angles 1–1.5 mm toward the left ear from the sagittal suture and moved toward the rostral direction until it reached the coronal suture. The dose solution was injected at a volume of 10 μ l/site, regardless of body weight.

2.3.2. Morphine-induced scratching behavior

Intracisternal administration of morphine was modified from the one devised (Ueda et al., 1979). A 30-gauge needle was bent (at 3.5 mm from the tip of the needle, at an angle of 40°) with a pair of pliers to form a J-shape, and then attached to a microsyringe. Morphine (0.3 nmol) was taken into the microsyringe and injected into the cerebello-medullary cistern (cisterna magna) of each mouse in a volume of 5 μ l/site. For administration, each mouse was

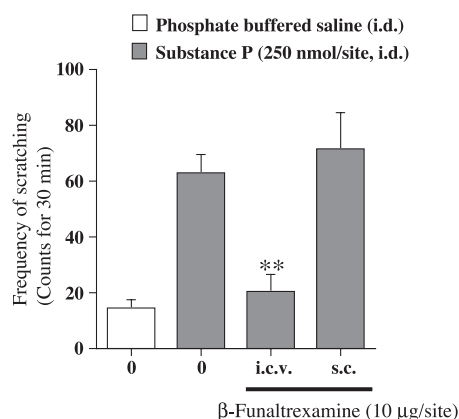


Fig. 1. Effect of μ -opioid receptor antagonist on the substance P-induced scratching behavior in mice. The ddY mouse was given an administration of subcutaneous (s.c.) or intracerebroventricular (i.c.v.) β -funaltrexamine, and 24 h later, substance P (250 nmol/site) or phosphate-buffered saline was injected intradermally. Immediately after the injection of pruritogen, the behavior was recorded for 30 min. Ordinate represents the frequency of scratching for 30 min (means and S.E.M.). $n=8-10$. ** $P<0.01$ when compared with the vehicle-treated group (the second column) and β -funaltrexamine-treated groups by Dunnett's test.

kept with the neck bent, and the needle was inserted into the atlanto-occipital gap. When the animal kept taking an abnormal posture after the administration, the animal was excluded from the experiment. The test compound, TRK-820 (1.25, 2.5, 5, 10 μ g/kg, s.c., – 30 min) or ketotifen (0.01, 0.1, 1, 10 mg/kg, i.p., – 30 min), was administered before the morphine injection. All scratches at a site of body by a hind paw were counted for 60 min after the injection of pruritogen.

2.4. Evaluation of the spontaneous motor activity

Spontaneous motor activity was evaluated by the Super-mex (CompACT AMS ver.3.41, Muromachi Kikai, Tokyo,

Japan) according to the method described (Masuo et al., 1997). Mice were individually placed in the transparent acrylic cage ($25 \times 42 \times 18$ cm in size) at the 30 min after the TRK-820 (0.3, 1, 3, 10 μ g/kg, s.c.) injection and measured the spontaneous motor activity for 60 min. Motor activity measurements were carried out between 1300 and 1700 h.

2.5. Data analysis

Statistical significance was analyzed as follows. When the variances were not homogeneous among the groups with Bartlett's test, a rank test was carried out by Kruskal–Wallis test. If a significant difference was detected among the groups, one-way analysis of variance was carried out to confirm the significant difference. In addition, multiple comparisons between the test substance-treated groups and the vehicle-administered group were carried out by Dunnett's test. On the other hand, when the variance were homogeneous among the groups in Bartlett's test, these were analyzed by one-way analysis of variance and Dunnett's test. For the statistical analysis, JMP ver 3.1J (SAS Institute) was used.

3. Results

3.1. Effect of μ -opioid receptor antagonist on the substance P-induced scratching behavior

The effects of μ -opioid receptor antagonist β -funaltrexamine on the substance P-induced scratching behavior are shown in Fig. 1. Twenty-four hours after the administration of β -funaltrexamine (s.c. or i.c.v.), substance P was intradermally injected and the number of scratches was counted for 30 min. β -Funaltrexamine at the dose of 10 μ g/site

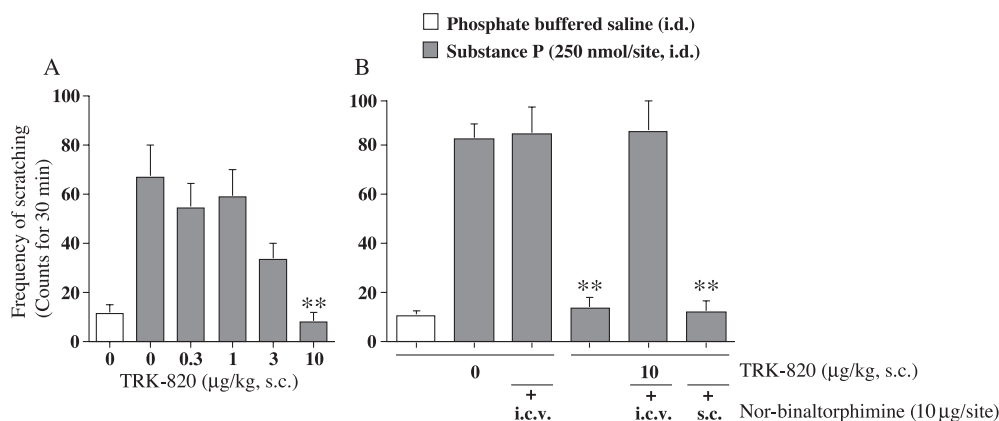


Fig. 2. Effect of TRK-820 and the antagonism of nor-binaltorphimine on the substance P-induced scratching behavior in mice. The ddY mouse was given an administration of subcutaneous (s.c.) TRK-820, and 30 min later, substance P (250 nmol/site) or phosphate-buffered saline was injected intradermally. Immediately after the injection of pruritogen, the behavior was recorded for 30 min (A). Nor-binaltorphimine was injected to the ddY mouse by intracerebroventricular (i.c.v.) or subcutaneous (s.c.) routes, and 24 h later, the scratching inhibitory activity of TRK-820 was estimated as A (B). Ordinate represents the frequency of scratching for 30 min (means and S.E.M.). $n=8-10$. ** $P<0.01$ when compared with the vehicle-treated group (the second column) and the test compounds-treated groups by Dunnett's test (A, B).

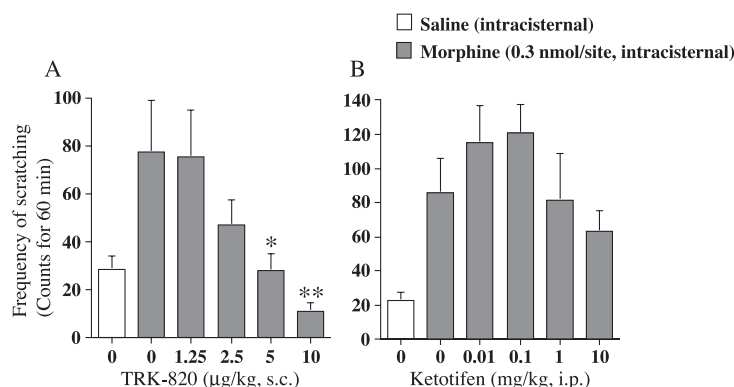


Fig. 3. Effect of TRK-820 or ketotifen on the morphine-induced scratching behavior in mice. The ddY mouse was given an administration of subcutaneous (s.c.) TRK-820 (A) or intraperitoneal (i.p.) ketotifen (B), and 30 min later, morphine (0.3 nmol/site) or saline was injected intracisternally. Immediately after the injection of pruritogen, the behavior was recorded for 60 min. Ordinate represents the frequency of scratching for 60 min (means and S.E.M.). $n = 10-15$. * $P < 0.05$, ** $P < 0.01$ when compared with the vehicle-treated group (the second column) and the test compounds-treated groups by Dunnett's test (A, B).

(i.c.v.) significantly inhibited the scratching behavior. But the subcutaneous administration of β -funaltrexamine at the same dose did not inhibit the scratching behavior.

3.2. Effect of κ -opioid receptor agonist TRK-820 on the substance P-induced scratching behavior and the antagonism of it by nor-binaltorphimine

The effects of TRK-820 on the substance P-induced scratching behavior are shown in Fig. 2. Thirty minutes after the subcutaneous administration of TRK-820, substance P was intradermally injected and the number of scratches was counted for 30 min. TRK-820 inhibited the scratching behavior dose-dependently and statistically significant inhibition was seen at the dose of 10 µg/kg (Fig. 2A). The inhibitory effect of TRK-820 was completely antagonized by the pretreatment with nor-binaltorphimine (i.c.v.) at the dose of 10 µg/site, which by itself did not affect the induction of scratches (Fig. 2B). On the other

hand, the subcutaneous administration of nor-binaltorphimine at the same dose failed to antagonize the scratching inhibitory activity of TRK-820 (Fig. 2B).

3.3. Effect of TRK-820 and ketotifen on the morphine-induced scratching behavior

The effects of TRK-820 or ketotifen on the morphine-induced scratching behavior are shown in Fig. 3. Thirty minutes after the administration of TRK-820 (s.c.) or ketotifen (i.p.), morphine was intracisternally injected and the number of scratches was counted for 60 min. TRK-820 inhibited the scratching behavior dose-dependently and statistically significant inhibition was observed at the doses of 5 and 10 µg/kg (Fig. 3A). On the other hand, ketotifen tended to increase the scratching behavior at lower doses (0.01 and 0.1 mg/kg) and to decrease at the highest dose (10 mg/kg). However, no significant difference was observed in any group (Fig. 3B).

3.4. Effect of TRK-820 on the spontaneous motor activity

The effect of TRK-820 on the spontaneous motor activity was shown in Fig. 4. Thirty minutes after the subcutaneous administration of TRK-820, the spontaneous motor activity was measured for 60 min using Supermex. TRK-820 showed no apparent inhibition of spontaneous motor activity at the dose up to 10 µg/kg.

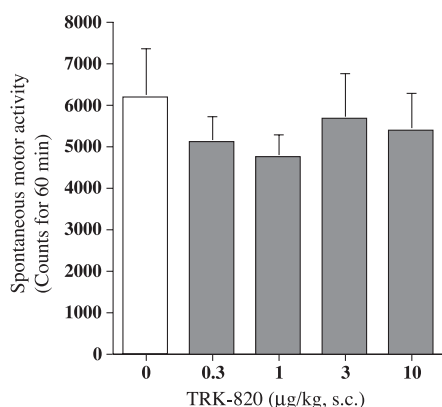


Fig. 4. Effect of TRK-820 on the spontaneous motor activity in mice. The ddY mouse was given an administration of subcutaneous (s.c.) TRK-820. Thirty minutes later, the mice placed in cage and measured the spontaneous motor activity for 60 min. Ordinate represents the counts of the spontaneous motor activity by the Supermex for 60 min (means and S.E.M.). $n = 7-8$.

4. Discussion

In the present studies, it was revealed that the activation of central μ -opioid system was involved in the scratching behaviors and the activation of central κ -opioid system antagonized the μ -opioid receptor mediated itch processing.

There are several reports that describe the effectiveness of μ -opioid receptor antagonists for the treatment of antihista-

mine-resistant pruritus in human (Monroe, 1989; Peer et al., 1996; Bergasa et al., 1999). Also in animals, it was reported that μ -opioid receptor antagonist naloxone inhibited the scratching behavior induced by the intradermal injection of substance P (Andoh et al., 1998), an endogenous tachykinin NK₁ receptor ligand recognized as the peripheral potent pruritogen in human (Hagermark et al., 1978). We have also confirmed that naltrexone (>0.03 mg/kg), an orally active μ -opioid receptor antagonist significantly inhibited the intradermal substance P-induced scratching behavior in ddY mice (data not shown). But the importance of the peripheral or central μ -opioid receptors remains controversial. Some reports suggested the importance of peripheral μ -opioid receptors in the itch processing. For example, loperamide, a peripheral restricted μ -opioid receptor antagonist, is shown to inhibit the compound 48/80 induced scratching behavior in mice (DeHaven-Hudkins et al., 2002). In addition, the involvement of μ -opioid receptor 1A on sensory nerve fibers in the transmission of pruritus is suggested (Stander et al., 2002). In contrast, there are reports which indicate the central μ -opioid receptor is the major participant in the processing of itch sensation. For example, naloxone reduced the itch intensity without effect on the vascular histamine reactions in a clinical experiment (Heyer et al., 1997). Another example is that naltrexone suppresses the scratching behavior in Nishiki Cinnamon (NC) mice with chronic dermatitis without affecting the cutaneous nerve activities (Maekawa et al., 2002). In our hand, the substance P-induced scratching behavior was suppressed by the central administration of β -funaltrexamine, a selective μ -opioid receptor antagonist (Portoghese et al., 1980), but not by the peripheral administration at the same dose (Fig. 1). The intracerebroventricular pretreatment by β -funaltrexamine is already known to inactivate the central μ -opioid receptor in mice (Narita et al., 1993). These results indicate that central μ -opioid receptors play an important role for the processing of peripheral itch stimuli in this case.

We have already reported that peroral TRK-820 a κ -opioid receptor agonist has the ability to suppress substance P-induced scratching behavior in mice via κ -opioid receptor mediated mechanism (Togashi et al., 2002). In the present study, it was shown that TRK-820 had scratching inhibitory activity by a subcutaneous administration (Fig. 2A) without apparent gross behavior change (Fig. 4), confirming the antipruritic activity of TRK-820. The intracerebroventricular pretreatment with nor-binaltorphimine (10 μ g/site), a selective κ -opioid receptor antagonist (Takemori et al., 1988), completely antagonized the inhibitory effect of TRK-820, but the subcutaneously administration did not at the same dose (Fig. 2B), suggesting that the antipruritic effect of systemically administered TRK-820 is in the CNS. It should be noted that intracerebroventricular pretreatment with nor-binaltorphimine (10 μ g/site) itself did not affect the scratching behavior.

To make the point clearer, we examined the effect of TRK-820 on the scratching behavior induced by the direct activation of central μ -opioid receptors. It is already

reported that the intracisternal administration of morphine to ddY mice causes the significant and naloxone-reversible scratching behavior (Tohda et al., 1997). Ketotifen, a typical antihistamine, did not suppress the morphine-induced scratching behavior (Fig. 3B), indicating that the scratching behavior was evoked by central mechanisms, not by mast cell degranulation by morphine as was suggested before (Barke and Hough, 1993). In the dose range that was employed in this study, ketotifen is known to significantly inhibit the increase in vascular permeability caused by passive cutaneous anaphylaxis (Inagaki et al., 1984), suggesting that ketotifen did not inhibit the scratching behavior induced by the administration of morphine to the central tissue even at doses sufficient for antiinflammatory effect. Therefore, the antihistamine ketotifen was considered ineffective in the treatment of pruritus mediated by morphine, which is supported by the clinical report (Duntelman et al., 1996). On the other hand, systemic administration of TRK-820 inhibited the frequency of morphine-induced scratching behavior (Fig. 3A), indicating that the κ -opioid receptor agonist was effective against pruritus induced by the activation of central μ -opioid receptors. It has been reported that activation of the κ -opioid receptor antagonizes various μ -opioid receptor mediated actions, for example, κ -opioid receptor agonists suppress the antinociceptive tolerance, rewarding effect and physical dependence on morphine (Pan, 1998). The antipruritic actions may be another example of the antagonism.

It is well known that the use of μ -opioid receptor antagonists against severe pruritus is limited in clinical use. One of the reasons is the side effects, e.g. pain, insomnia, depersonalization as reported for the patients with cholestatic liver disease (Bergasa et al., 1998). The second reason is the opioid withdrawal like symptom (Zylicz and Krajnik, 1999), which is considered to be the result from increased exposure of μ -opioid receptors to endogenous μ -opioid ligands in disease states (Thornton and Losowsky, 1988). The third reason is that they antagonized the antinociceptive activity of morphine when used to treat morphine-induced pruritus (Friedman and Dello Buono, 2001). In contrast, it is less likely that κ -opioid receptor agonists demonstrate the antipruritic activity with these events. κ -Opioid receptor agonists may ameliorate the pruritus caused by morphine without losing antinociceptive activity. However, κ -opioid receptor agonists are suspected to have different type of adverse effects. For example, some κ -opioid receptor agonists, spiradoline and enadoline have been discontinued in the clinical trials against post-surgical pain because of their severe central side effects such as dysphoria (Barber and Gottschlich, 1997). TRK-820 used in this study, however, has unique characteristics compared with the other κ -opioid receptor agonists as producing no gross behavioral change (Fig. 4), and showing different pharmacological properties such as weak aversive effect (Tsuji et al., 2001). Therefore, TRK-820 may be a useful compound to treat pruritus.

In clinical settings, antihistamine-resistant pruritus is more common and implicated in central opioidergic mechanism (Jones and Bergasa, 1999). This is supported by the clinical reports that opioid receptor antagonists are effective in some cases to intractable pruritus associated with cholestatic liver disease, chronic renal failure and atopic dermatitis (Bergasa et al., 1999; Monroe, 1989; Peer et al., 1996) which show higher serum concentration of endogenous opioid ligands to μ -opioid receptors (Thornton and Losowsky, 1988, 1991; Georgala et al., 1994). Our results indicate that TRK-820 exert its antipruritic activity by acting on the CNS, different from antihistamines, and antagonize the central μ -opioid receptor mediated itch processing, suggesting the possibility of the clinical application to those central origin itching.

In conclusion, central μ -opioid receptors play an important role to process the itch impulses both by central and peripheral pruritogen. The mechanism of systemic administration of κ -opioid receptor agonists on the antipruritic effect may be the blockage of the itch pathway by antagonizing the activation of central μ -opioid receptors.

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