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Title page

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Suppression of acute herpetic pain-related responses by the κ-opioid receptor agonist TRK-820 in mice

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Running title page

Running Title: TRK-820 effect on acute herpetic pain

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Abbreviations: ANOVA, analysis of variance; DRG, dorsal root ganglion; HSV-1, herpes simplex virus type-1; i.c.v., intracerebroventricular; i.pl., intraplantar; i.t., intrathecal; nor-BNI, norbinaltorphimine; p.o., per os; RT-PCR, reverse transcription and polymerase chain reaction; s.c., subcutaneous; TRK-820, (-)-17-cyclopropylmethyl-3,14β-dihydroxy-4,5α- epoxy-6β-[*N*-methyl-3-*trans*-3-(3-furyl) acrylamido] morphinan hydrochloride

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ABSTRACT

TRK-820 is a κ-opioid receptor agonist, which has pharmacological characteristics different from typical κ-opioid receptor agonists. This study was conducted to determine the antiallodynic and antihyperalgesic effects of TRK-820 in a mouse model of acute herpetic pain and to compare them with those of the κ-opioid receptor agonist enadoline and the μ-opioid receptor agonist morphine. Percutaneous inoculation with herpes simplex virus type-1 induced tactile allodynia and mechanical hyperalgesia in the hind paw on the inoculated side. TRK-820 (0.01–0.1 mg/kg, p.o.), enadoline (1–10 mg/kg, p.o.) and morphine (5–20 mg/kg, p.o.) dose dependently inhibited the allodynia and hyperalgesia, but the antiallodynic and antihyperalgesic dose of enadoline markedly decreased spontaneous locomotor activity. The antinociceptive action of TRK-820 (0.1 mg/kg) was completely antagonized by pretreatment with norbinaltorphimine, a κ-opioid receptor antagonist, but not by naltrexone, a μ-opioid receptor antagonist. Repeated treatment with morphine (20 mg/kg, four times) resulted in the reduction of antiallodynic and antihyperalgesic effects, while the inhibitory potency of TRK-820 (0.1 mg/kg) was almost the same even after the fourth administration. There was no cross-tolerance in antinociceptive activities between TRK-820 and morphine. Intrathecal and intracerebroventricular, but not intraplantar, injections of TRK-820 (10-100 ng/site) suppressed the allodynia and hyperalgesia. These results suggest that TRK-820 inhibits acute herpetic pain through κ-opioid receptors in the spinal and

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supraspinal levels. TRK-820 may have clinical efficacy in acute herpetic pain with enough safety margins.

Herpes zoster is caused by the re-activation of latent varicella-zoster virus in the sensory ganglia and characterized by neurocutaneous symptoms, such as the rash of blisters and severe pain, including allodynia and hyperalgesia and spontaneous pain (Loeser, 1986; Portenoy et al., 1986; Nurmikko et al., 1990). Early treatment of herpes zoster with antiviral agents, such as acyclovir and vidarabine, shortens the duration of skin lesions and complications related to herpes zoster (Guann, 1994). However, these agents do not promptly relieve acute herpetic pain (Wood et al., 1994; Lancaster et al., 1995). Nonsteroidal anti-inflammatory drugs such as diclofenac and antidepressants such as amitriptyline are used for the treatment of the pain of herpes zoster, but these medications do not always relieve severe herpetic pain (Loeser, 1986; Dworkin and Portenoy, 1996). Therefore, new potent analgesics with few adverse effects are desired.

Recently, we have developed a mouse model of acute herpetic pain; cutaneous inoculation with herpes simplex virus type-1 (HSV-1) causes herpes zoster-like lesions on the skin of inoculated dermatome, allodynia (aversive responses to innocuous tactile stimulation) and hyperalgesia (aversive responses to noxious mechanical stimulation) (Takasaki et al., 2000a). The pain-related responses and eruption become apparent on day 5 after inoculation, a day when HSV-1 proliferates in the dorsal root ganglion (DRG) (Takasaki et al., 2000b). When started before viral replication in the DRG, repeated treatment with anti-HSV-1 agent acyclovir inhibits the appearance of allodynia and hyperalgesia. In contrast, when started from

day 5 after inoculation, acyclovir treatment does not affect allodynia and hyperalgesia (Takasaki et al., 2000a).

Single injection of morphine, a μ-opioid receptor agonist, dose dependently attenuates the herpes infection-induced allodynia and hyperalgesia (Takasaki et al., 2000b). However, tolerance rapidly develops after repeated administration (Takasaki et al., 2001). It has not been clear whether κ-opioid receptor agonists inhibit allodynia and hyperalgesia in this model, although they have shown to have antinociceptive effects in various transient nociceptive tests. In chronic pain models, suppressive effects of κ-opioid receptor agonists on allodynia and hyperalgesia and the site of action remain controversial. Systemic administration of the κ-opioid receptor agonist enadoline inhibits allodynia and hyperalgesia in a rat model of surgical pain (Field et al., 1999). Asimadoline, a peripherally-selective κ-opioid receptor agonist, produces antinociception in rats given the loose constriction of the sciatic nerve (Walker et al., 1999). With regard to intrathecal injection, GR89696, a putative κ_2-opioid receptor agonist, suppresses hyperalgesia and allodynia in rats with peripheral neuritis or neuropathy (Eliav et al., 1999). On the other hand, the prototype κ-opioid receptor agonist U-50,488H does not produce antiallodynic effect in rats given spinal nerve ligation, and in spinally injured rats (Lee et al., 1995; Hao et al., 1998).

TRK-820, (-)-17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[*N*-methyl-3-*trans*-3-(3- furyl) acrylamido] morphinan hydrochloride, is a potent κ-opioid receptor agonist

(Nagase et al., 1998). TRK-820 has some pharmacological characteristics different from the typical κ-opioid receptor agonists. Unlike U-50,488H or enadoline, TRK-820 does not produce any sedative effect at antinociceptive doses in mice (Endoh et al., 1999). The important merit of TRK-820 is that it does not produce any significant place aversion or place preference in rodents (Tsuji et al., 2001), while other κ-opioid receptor agonists such as U-50,488H and ICI-199,441 do. Moreover, the development of morphine tolerance was suppressed by the co-administration of U-50,488H, but not TRK-820 (Tsuji et al., 2000). TRK-820 produces potent antinociception in mice acetic acid writhing test (Nagase et al., 1998) and in the paw pressure test in healthy and inflamed rats through κ-opioid receptors (Endoh et al., 2000). The present study was conducted to determine whether TRK-820 suppresses allodynia and hyperalgesia in mice with herpes zoster-like skin lesions. Additionally, alterations of κ-opioid receptors mRNA expression in dorsal horn and DRG in this model were investigated.

Materials and Methods

Animals. Female BALB/c mice weighing 18–20 g (6 week old at the start of experiment; Japan SLC, Shizuoka, Japan) were used. They were housed six per cage under controlled temperature (22 ± 1 °C) and humidity (55 ± 10%). The room was lighted form 7:00 AM to 7:00 PM and during the behavioral test. Food and water were freely available. HSV-1 inoculation and behavioral experiments were done in the infection room of the Molecular Gene Research Center, Toyama Medical and Pharmaceutical University. The study was approved by the Committee for Animal Experiments at Toyama Medical and Pharmaceutical University. Behavioral tests were performed according to the guidelines on ethical standards for investigations of experimental pain in animals (Zimmermann, 1983).

Virus inoculation. The mice were inoculated with HSV-1 as described (Takasaki et al., 2000a). In brief, the midflank and right foot was clipped and depilated with a chemical depilatory; and three days later, HSV-1 (7401H strain, 1×10^6 plaque-forming units in 10 µl) was inoculated on the scarified skin (5 × 5 mm) of the right hind paw. The contralateral hind paw was without inoculation.

Evaluation of pain-related responses. Pain-related responses of the hind paw were assessed as described (Takasaki et al., 2000a). The mice were placed individually in an

acrylic cage (11 × 18 × 15 cm, W × D × H) with a wire mesh bottom. After at least 15-min acclimation period, von Frey filaments with a bending force of 0.17 g (innocuous stimulation) or 1.20 g (noxious stimulation) were pressed perpendicularly against the plantar skin and held for 3–5 s with it slightly buckled. The responses to these stimuli were ranked as follows: 0 = no response; 1 = move away from von Frey filament; 2 = immediate flinching or licking of the hind paw. The stimulation of the same intensity was applied six times to each hind paw at intervals of several seconds. The mean score of six-time stimulation served as pain-related score. All normal mice tested responded at least three times to six-time stimulation with the filament of 1.20 g strength and showed no responses to the filament of 0.17 g strength.

The percentage of analgesic effect of drugs used in this study was calculated as follows:

$$\text{Analgesic effect (\%)} = \frac{\text{PS (inoculated side, vehicle)} - \text{PS (inoculated side, drug)}}{\text{PS (inoculated side)} - \text{PS (contralateral side)}} \times 100$$

where PS is pain-related score.

Drug administration. TRK-820 and enadoline were synthesized in Toray Industries. TRK-820, enadoline and morphine hydrochloride (Sankyo, Tokyo, Japan) were dissolved in distilled water and injected per os (p.o.) with a bulb needle. For intraplantar (i.pl.), intrathecal (i.t.) and intracerebroventricular (i.c.v.) injections, TRK-820 was dissolved in physiological

saline. Effects of these opioids were generally tested on day 6 and in two experiments on day 7 post-inoculation. Norbinaltorphimine (nor-BNI; synthesized in Toray Industries) and naltrexone (Sigma, St. Louis, MO) were dissolved in physiological saline. Nor-BNI (20 mg/kg) and naltrexone (1 mg/kg) were administered subcutaneously (s.c.) 24 h and 15 min before TRK-820 administration, respectively. These dosing regimens for nor-BNI and naltrexone specifically antagonize κ-opioid and μ-opioid action, respectively (Endoh et al., 1992; Takasaki et al., 2001). The dosage is given in terms of the weight of salt.

Measurement of spontaneous locomotor activity. Mice were put individually into an acrylic cage (26 × 16 × 13 cm, W × D × H) and spontaneous locomotor activity was measured with Supermex® (Muromachi Kikai, Tokyo, Japan), which monitors infrared ray radiated from the animal's body with pyroelectric detectors. The system can monitor vertical and horizontal movements, including locomotion, rearing and head movement (Masuo et al., 1997), all of which were counted as locomotor activity. Measurement was done for 60 min after opioid administration between 9:00 AM and 7:00 PM.

Reverse transcription and polymerase chain reaction (RT-PCR). After decapitation under ether anesthesia, the DRGs at the L4 and L5 levels and lumbar enlargement were rapidly removed from mice. DRGs and lumbar dorsal horn on the

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inoculated side were stored at -80 °C until assay. Total RNA was extracted from the samples using RNAzol™ B (TEL-TEST, Inc., Friendswood, TX) and treated with DNase I (Takara, Kyoto, Japan) at 37 °C for 30 min. After extraction with phenol-chloroform and ethanol, it was reverse transcripted. RT reaction mixture (1 μl) was used for PCR amplification. The sequences of primers for κ-opioid receptor were 5'-tttatcctggtggaggctctggga-3' (forward) and 5'-ctcatggaagcaggatcctgaact-3' (reverse), which give a 236 bp PCR product. Primers for glyceraldehyde 3-phosphate dehydrogenase (G3PDH) were 5'-caaaggtcatccatgacaac-3' (forward) and 5'-ttactccttggaggccatgt-3' (reverse), which give a 527 bp product. Reaction products were separated on a 1.5% agarose gel and stained with ethidium bromide. To determine the expression levels the density of the bands were measured with a densitometer (DensitoGraph, ATTO, Tokyo, Japan).

Statistical analysis. Unless otherwise mentioned, the means of data are presented together with standard errors of the means (SEM). Data on the time course of analgesic effects were analyzed with the Friedman repeated-measures analysis of variance (ANOVA) on ranks with *post hoc* Dunnett's test. Statistical differences between different groups were analyzed with Kruskal-Wallis one-way analysis of variance on ranks with *post hoc* Dunnett's test (multiple comparisons versus a control) or Dunn's test (all pairwise comparisons). A value of $P < 0.05$ was considered significant.

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Results

Effects of κ- and μ-opioid receptor agonists on acute herpetic pain-related responses. Mice given HSV-1 inoculation show allodynia (responses to von Frey filament of 0.17-g strength) and hyperalgesia (responses to von Frey filament of 1.20-g strength) on day 5 after inoculation and these responses are marked from day 6 to at least day 8 (Takasaki et al., 2000a, 2002). In this experiment, therefore, opioid agonists were administered on day 6 after inoculation. TRK-820 (0.01–0.1 mg/kg, p.o.) produced the significant (Dunnett's test) and dose-dependent inhibition of pain-related responses of HSV-1-inoculated hind paw to the filaments of 0.17 or 1.20 g strength (Fig. 1A and B). The highest dose of 0.1 mg/kg almost completely relieved both allodynia and hyperalgesia. The effects peaked 30 min after administration and subsided by 2–3 h. TRK-820 did not affect the pain-related responses of the contralateral (uninoculated) hind paw (data not shown).

Enadoline at oral doses of 3 and 10 mg/kg, but 1 mg/kg, markedly and significantly (Dunnett's test) inhibited pain-related responses of the inoculated hind paw (Fig. 1C and D); pain-related responses to the filament of 1.20 g strength were almost abolished by the doses of 3 and 10 mg/kg (Fig. 1D). The doses of 3 and 10 mg/kg also suppressed the responses of the contralateral hind paw to the filament of 1.20 g strength (data not shown).

Morphine (5–20 mg/kg, p.o.) produced the significant (Dunnett's test) and dose-dependent inhibition of allodynia and hyperalgesia; however, the highest dose tested (20

mg/kg) did not produce complete inhibitions (Fig. 1E and F). The effects peaked 15–30 min after administration and subsided by 60–90 min.

TRK-820 (0.01–0.1 mg/kg, p.o.) did not affect the spontaneous locomotor activity of healthy mice for 1 h after administration (Fig. 2). Enadoline at oral doses of 3 and 10 mg/kg produced marked and significant (Dunnett's test) decrease in locomotor activity (Fig. 2). Some mice showed motor paralysis in the forelimbs and hindlimbs, which became apparent within 30 min and persisted at least for 4 h (data not shown). Although there was an increased tendency of locomotor activity after enadoline at a dose of 1 mg/kg, the effect was not statistically significant (Fig. 2). The experimenter had an impression that morphine at an oral dose of 20 mg/kg produced a slight increase in locomotor activity during the assessment of pain-related responses. However, morphine (5–20 mg/kg) did not affect the locomotor activity in healthy mice (Fig. 2).

Effects of opioid receptor antagonists on the action of TRK-820. The inhibition of herpetic pain-related responses by TRK-820 (0.1 mg/kg, p.o.) was significantly (Dunn's test) blocked by pretreatment with nor-BNI (20 mg/kg, s.c., -24 h), but not naltrexone (1 mg/kg, s.c., -15 min) (Fig. 3). On the contrary, the inhibitory action of morphine (20 mg/kg, p.o.) was markedly blocked by naltrexone (1 mg/kg), but not by nor-BNI (20 mg/kg) (Fig. 3).

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Effects of local injections of TRK-820. HSV-1-induced allodynia and hyperalgesia were not attenuated by i.pl. injections of TRK-820 (10–100 ng/site) into the sole, the site of filament stimulation (Fig. 4). I.t. injections of TRK-820 (10–100 ng/site) produced the potent and dose-dependent inhibition of allodynia and hyperalgesia; the dose of 100 ng/site produced a complete inhibition (Fig. 4). No behavioral alterations (increase or decrease of locomotion) were observed after the doses tested. I.c.v. injection of TRK-820 (10-100 ng/site) also produced dose-dependent inhibition; however, the effect was weaker than that of i.t. injection.

Repeated administration of TRK-820 and morphine. Repeated administration of TRK-820 (0.1 mg/kg, p.o., twice a day) produced a constant inhibition of allodynia and hyperalgesia for at least 2 days; the effects of the fourth administration were not significantly different from those of the first administration (Fig. 5A). The inhibitory effects of morphine (20 mg/kg, p.o., twice a day) rapidly decreased after repeated administration. The effects of the third and fourth administration were significantly weaker than those of the first administration (Fig. 5B).

Pretreatment with morphine (20 mg/kg, p.o., three times) markedly reduced the inhibitory actions of morphine at a oral dose of 20 mg/kg, without effects on the inhibitory actions of

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TRK-820 at a oral dose of 0.1 mg/kg (Fig. 6).

κ-Opioid receptor mRNA expression. HSV-1 inoculation did not affect the expression levels of κ-opioid receptor mRNA in the DRGs (L4 and L5) on the inoculated side on day 6 after inoculation; the levels of κ-opioid receptor mRNA normalized to the level of G3PDH mRNA in naive and inoculated mice were 0.77 ± 0.11 and 0.75 ± 0.08 *(n* = 6 each), respectively. HSV-1 inoculation also did not affect the level of κ-opioid receptor mRNA in the lumbar dorsal horn; the normalized levels of κ-opioid receptor mRNA in naive and inoculated mice were 1.51 ± 0.6 and 1.61 ± 0.13 (n = 6 each), respectively.

Discussion

The κ-opioid receptor agonist TRK-820 (0.01–0.1 mg/kg) produced the marked inhibition of allodynia and hyperalgesia in mice with acute herpetic pain, without effects on spontaneous locomotor activity. The degree of antiallodynic and antihyperlagesic effects of TRK-820 (0.03 mg/kg) was similar to that of morphine (20 mg/kg), and the duration of the former was twice as long as that of the latter. The results are consistent with other study, in which the antinociceptive effects of TRK-820 were more potent than those of morphine and the κ-opioid receptor agonists U-50,488H, ICI-199,441 and enadoline in various acute nociceptive tests (Endoh et al., 1999). It is possible that TRK-820 is effective against acute herpetic pain in humans. Enadoline, another κ-opioid receptor agonist tested, also inhibited HSV-1-induced allodynia and hyperalgesia in mice, but it almost abolished the pain-related responses only at doses that markedly suppressed locomotor activity. Therefore, it is possible that the inhibition by enadoline of pain-related responses were due to the suppression of motor functions.

Acute tolerance did not develop to the inhibitory effect of TRK-820 on herpetic pain-related responses, whereas tolerance rapidly developed to the inhibitory effect of morphine. In addition, TRK-820 showed no cross-tolerance to morphine. Tolerance develops to the atypical μ-opioid receptor agonist methadone, although more slowly than to morphine, and partial cross-tolerance develops between methadone and morphine in rats with ischemic nerve injury (Bulka et al., 2002). Morphine produces cross-tolerance to δ-opioid receptor

agonists but not to κ-opioid receptor agonists in rats with neuropathy due to sciatic nerve injury (Catheline et al., 1996; Walker et al., 1999). Thus, TRK-820 has opioid agonistic activity different from μ- and δ-opioid receptor agonists. TRK-820 could be useful even in patients with opioid tolerance.

The antiallodynic and antihyperalgesic effects of TRK-820 were almost completely antagonized by the selective κ-opioid receptor antagonist nor-BNI at a dose that did not affect the effects of morphine. On the other hand, the effects of TRK-820 were not inhibited by the selective μ-opioid receptor antagonist naltrexone at a dose that almost abolished the effects of morphine. The results are consistent with other studies, in which the antinociceptive effects of TRK-820 were suppressed by nor-BNI but not by naloxone in normal rodents (Endoh et al., 1999, 2000). The selective and potent affinity of TRK-820 for κ-opioid receptors has been shown by in vitro studies using mouse vas deferens, guinea pig ileum and Chinese hamster ovary expressing recombinant rat μ-, δ-, or κ-opioid receptors (Nagase et al., 1998; Seki et al., 1999). Thus, it is suggested that TRK-820 relieves acute herpetic pain mainly through κ-opioid receptor.

TRK-820 did not affect locomotor activity and motor functions at the doses that inhibited pain-related responses. On the other hand, enadoline inhibited pain-related responses and locomotor activity at the same doses. The ratio of sedative dose to antinociceptive dose of TRK-820 is larger than other κ-opioid receptor agonists U-50,488H and ICI-199,441 (Endoh

et al., 1999). TRK-820 does not produce any significant place aversion and place preference in mice, while they occur after the other κ-opioid receptor agonists (Tsuji et al., 2001). The reason why TRK-820 has less sedative effect than the other κ-opioid receptor agonists remains unclear. Considering that enadoline and U-50,488H have a selective affinity for κ_1-opioid receptors (Butelman et al., 1998), other receptors than κ_1-subtype may be also involved in the pharmacological actions of TRK-820.

With regard to central action, i.t. TRK-820 (10–100 ng/site) produced a marked inhibition of allodynia and hyperalgesia. Given that the average body weight of mice was 20 g, a local dosage of 100 ng/site is equivalent to .005 mg/kg, which was much smaller than the oral equipotent dose (0.1 mg/kg). Therefore, the spinal cord may play an important role in the antiallodynic and antihyperalgesic effects of oral TRK-820. Although the effects of TRK-820 were smaller after i.c.v. injection than after i.t. injection, i.c.v. injections of TRK-820 (10–100 ng/site) produced dose-dependent inhibitions. Thus, although to a lesser extent, the brain action may be also involved in the effects of oral TRK-820.

κ-Opioid receptor-like immunoreactivities are localized in the superficial dorsal horn and dorsal root ganglion cells (Ji et al., 1995). Dorsal rhizotomy produces a significant reduction in the binding of [^{3}H] U-69,593, a κ_1 subtype selective ligand, in the spinal dorsal horn (Stevens and Seybold, 1995). Additionally, κ-opioid receptor mRNA is intensely expressed in the superficial dorsal horn (Maekawa et al., 1994). κ-Opioid receptors present in the central

terminals of primary sensory neurons and dorsal horn neurons in the spinal cord may be involved in the antiallodynic and antihyperalgesic effects of TRK-820. In the case of other κ-opioid receptor agonists, effects on allodynia and/or hyperalgesia and the site of the actions remain unclear. Neither i.c.v. nor i.t. U-50,488H, well-defined as a κ-opioid receptor agonist, attenuates allodynia in the Chung model (Lee et al., 1995). I.t. U-50,488H did not alleviate chronic allodynia-like behavior in the central pain model using spinally injured rats (Hao et al., 1998). On the other hand, i.t. GR89,696, a putative κ_2-opioid receptor agonist, blocked hyperalgesia and allodynia in both the neuritis model and chronic constriction injury models (Eliav et al., 1999). The suppressive effect of κ-opioid receptor agonists on allodynia or hyperalgesia, and the site of the actions may depend on animal model of pain, the kind of stimulus and its intensity, and compounds used.

With regard to the periphery, an i.pl. injection of TRK-820 (10–100 ng/site) did not affect pain-related responses of mice given HSV-1 inoculation, suggesting that peripheral κ-opioid receptors are not involved in the effect of TRK-820. Conversely, i.pl. injections of κ-opioid receptor agonists, asimadoline and ICI204448, are reported to inhibit pain-like behaviors in sciatic nerve-injured rats (Keïta et al., 1995; Walker et al., 1999). With these findings taken into account, the present result raises the possibility that the roles of κ-opioid receptors in pain-related pathology are different between herpetic infection and the surgical injury of the sensory nerve. In this context, expression of κ-opioid receptor mRNA increased in the DRG

on the affected side in mice with allodynia after the transection of the spinal nerve (Sung et al., 2000), though, in this study, HSV-1 inoculation did not affect the expression level of κ-opioid receptor mRNA in the DRG. Persistent, peripheral inflammation increases κ-opioid receptor mRNA in the spinal dorsal horn (Maekawa et al., 1995) and decreases κ-opioid receptor-like immunoreactivity in the DRG (Sung et al., 2000). Thus, peripheral inflammation may affect the expression of κ-opioid receptors in the primary sensory neurons and dorsal horn neurons. On the other hand, HSV-1 inoculation did not affect the expression levels of κ-opioid receptor mRNA in the spinal dorsal horn and DRG in this experiment. κ-Opioid receptors may not play an important role in the development of acute herpetic pain.

In summary, TRK-820, a novel κ-opioid receptor agonist, produced the potent inhibition of acute herpetic pain without affecting the motor functions in mice. It may be worth testing the potency of TRK-820 to inhibit pain in patients with herpes zoster.

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Figure Legend

Fig. 1. Effects of oral administration of TRK-820, enadoline and morphine on allodynia and hyperalgesia induced by HSV-1 inoculation in mice. Mice were given HSV-1 inoculation on the unilateral hind paw; the contralateral hind paw (CL) was without inoculation. TRK-820 (0.01–0.1 mg/kg; A, B), enadoline (1–10 mg/kg; C, D), morphine (5–20 mg/kg; E, F) and vehicle (VH) were administered p.o. on day 6 after inoculation. Pain-related scores on the stimulation with filaments with a bending force of 0.17 (A, C, E; allodynia) and 1.20 g (B, D, F; hyperalgesia), respectively, were plotted against time after drug administration. The data presented are means and SEM *(n* = 6). **P* < 0.05 as compared with preadministration values (Dunnett's test after Friedman repeated-measures ANOVA on ranks).

Fig. 2. Effects of TRK-820, enadoline, and morphine on spontaneous locomotor activity in mice. TRK-820, enadoline, morphine, and vehicle (VH) were administered p.o. and spontaneous movements were counted for 60 min after medication. The data presented are means and SEM *(n* = 6). **P* < 0.05 as compared with vehicle group (Dunnett's test after Kruskal-Wallis one-way ANOVA on ranks).

Fig. 3. Effects of pretreatment with norbinaltorphimine (BNI) and naltrexone (NTX) on the inhibitory actions of TRK-820 and morphine on allodynia and hyperalgesia induced by

HSV-1 inoculation in mice. Allodynia (A) and hyperalgesia (B) were assessed 30 min after TRK-820 (0.1 mg/kg, p.o.) and morphine (20 mg/kg, p.o.) on day 6 after inoculation. BNI (20 mg/kg) and NTX (1 mg/kg) were injected s.c. 24 h and 15 min, respectively, before TRK-820 and morphine. VH, vehicle (saline). The data presented are means and SEM *(n* = 6). *P < 0.05 when compared with VH (Dunn's test after Kruskal-Wallis one-way ANOVA on ranks).

Fig. 4. Comparison of inhibitory effects of local injections of TRK-820 on allodynia and hyperalgesia induced by HSV-1 inoculation in mice. Mice were given an intraplantar (i.pl.), intrathecal (i.t.) or intracerebroventricular (i.c.v.) injection of TRK-820. Allodynia (A) and hyperalgesia (B) were assessed 30 min after injection. For comparison, the results 30 min after oral (p.o.) administration of TRK-820 were calculated from data of Fig. 1. The data presented are means and SEM *(n* = 6).

Fig. 5. Effects of repeated administration of TRK-820 and morphine on allodynia and hyperalgesia induced by HSV-1 inoculation in mice. TRK-820 (0.1 mg/kg; A, B) and morphine (20 mg/kg; C, D) were injected p.o. at 12-h intervals. Effects of TRK-820 and morphine on allodynia (A, C) and hyperalgesia (D, E) were assessed 30 and 15 min after administration, respectively. The data presented are means and SEM *(n* = 6). *P < 0.05 when compared with the first administration (Dunnett's test after Friedman repeated-measures

ANOVA on ranks).

Fig. 6. Effects of TRK-820 and morphine on HSV-1 inoculation-induced allodynia and hyperalgesia after repeated morphine administration in mice. The mice were given p.o. morphine (MOR; 20 mg/kg) or vehicle (VH) three times and then TRK-820 (0.1 mg/kg) or MOR (20 mg/kg); administration schedule was the same as that of Fig. 5. The effects of TRK-820 and morphine on allodynia (A) and hyperalgesia (B) were assessed 30 and 15 min after administration, respectively. *$P < 0.05$ as compared with VH (Dunn's test after Kruskal-Wallis one-way ANOVA on ranks)

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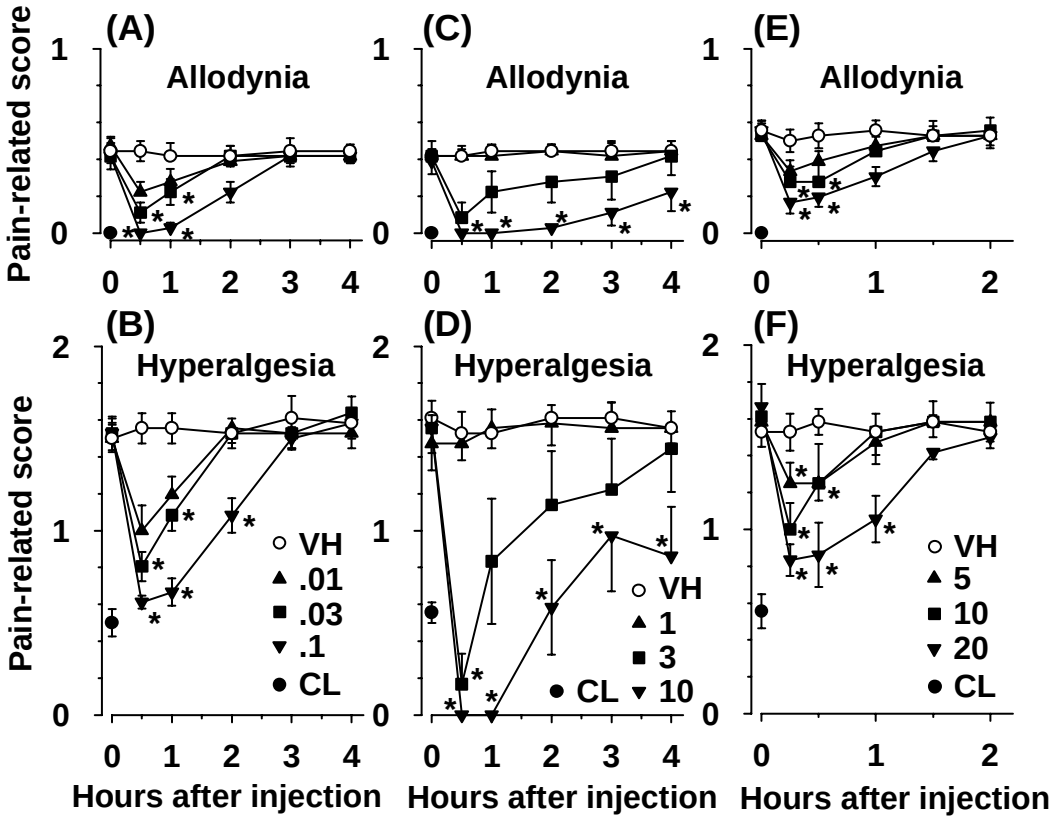
Fig. 1 (Takasaki et al.)



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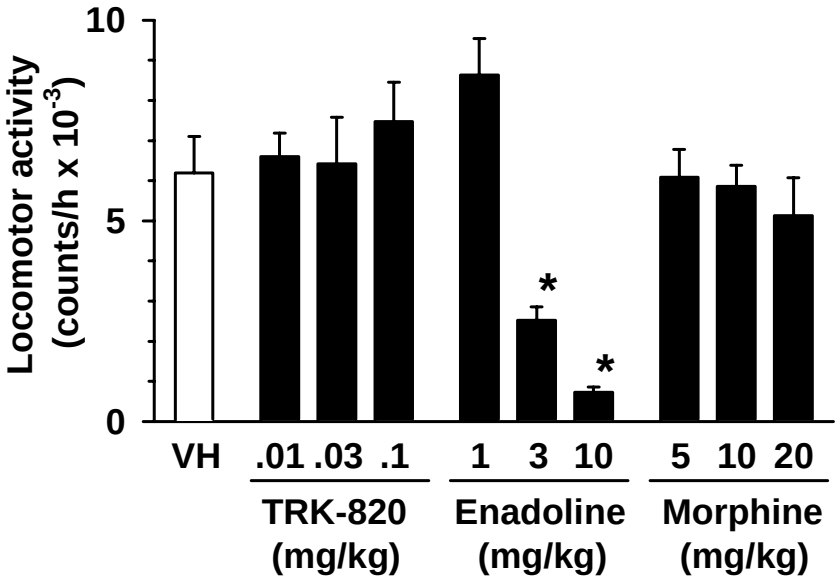

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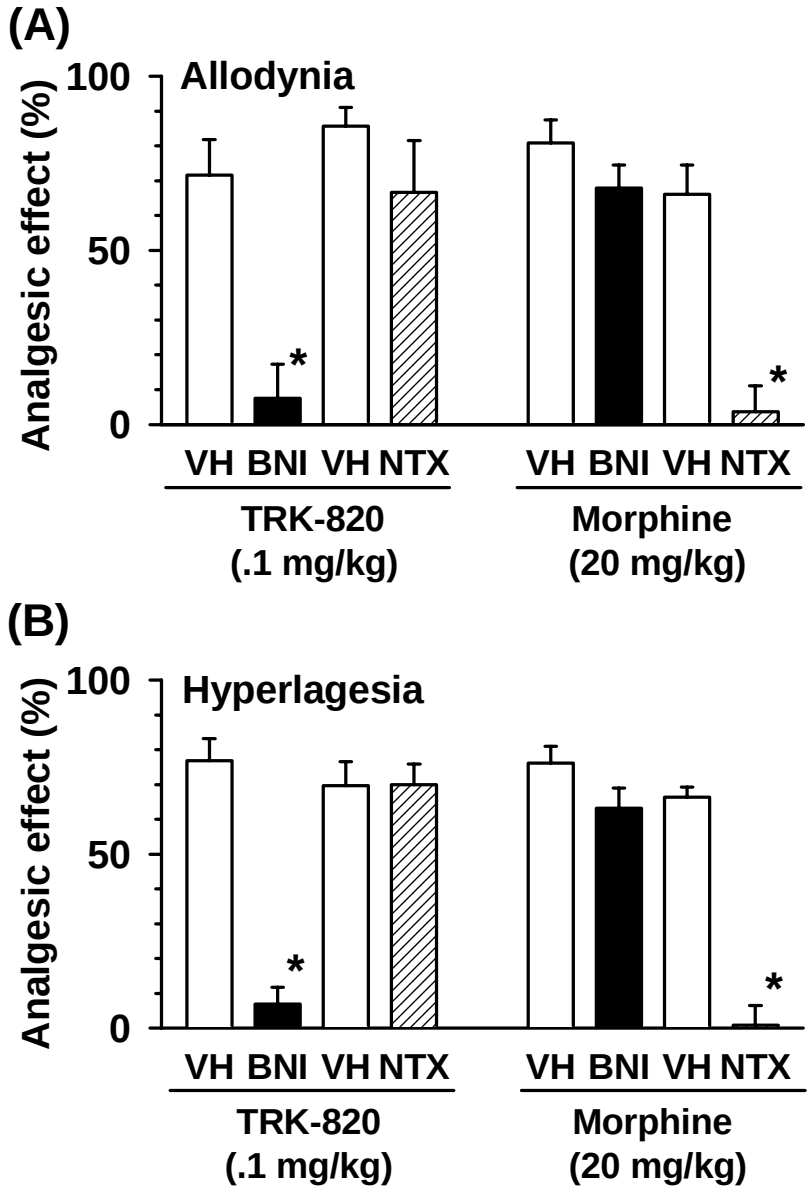

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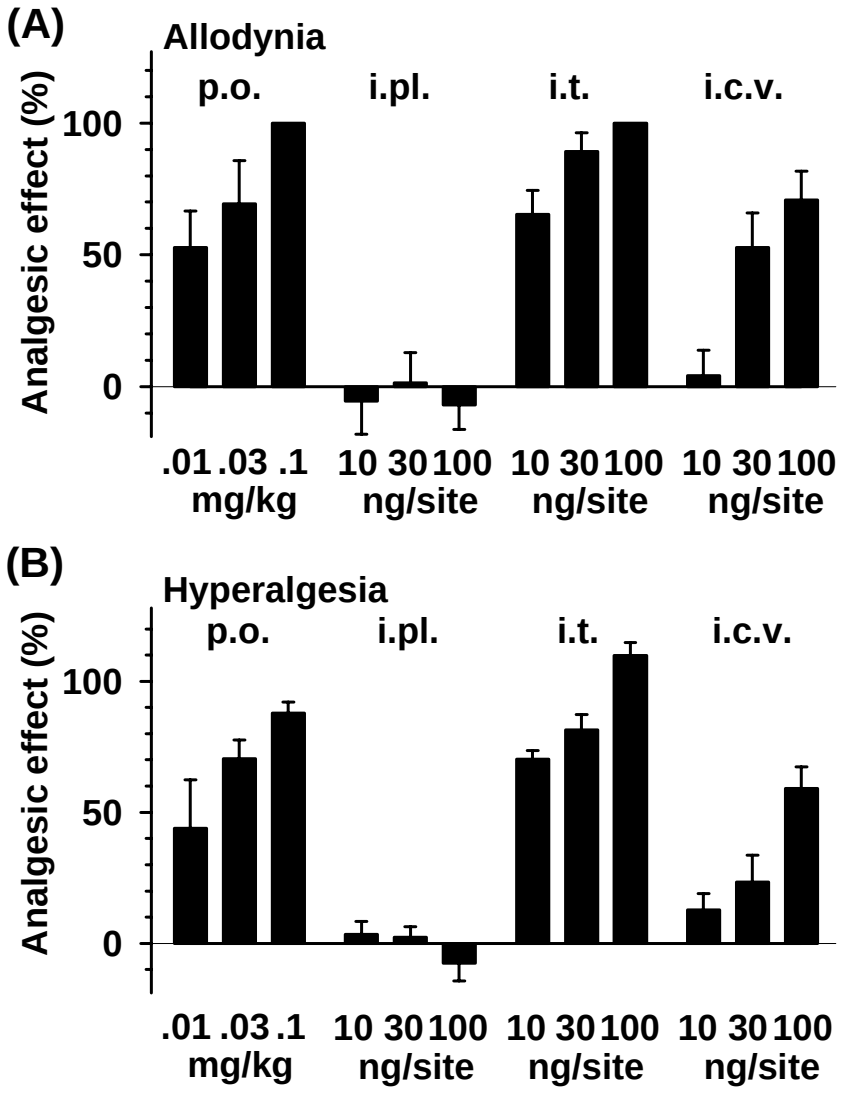

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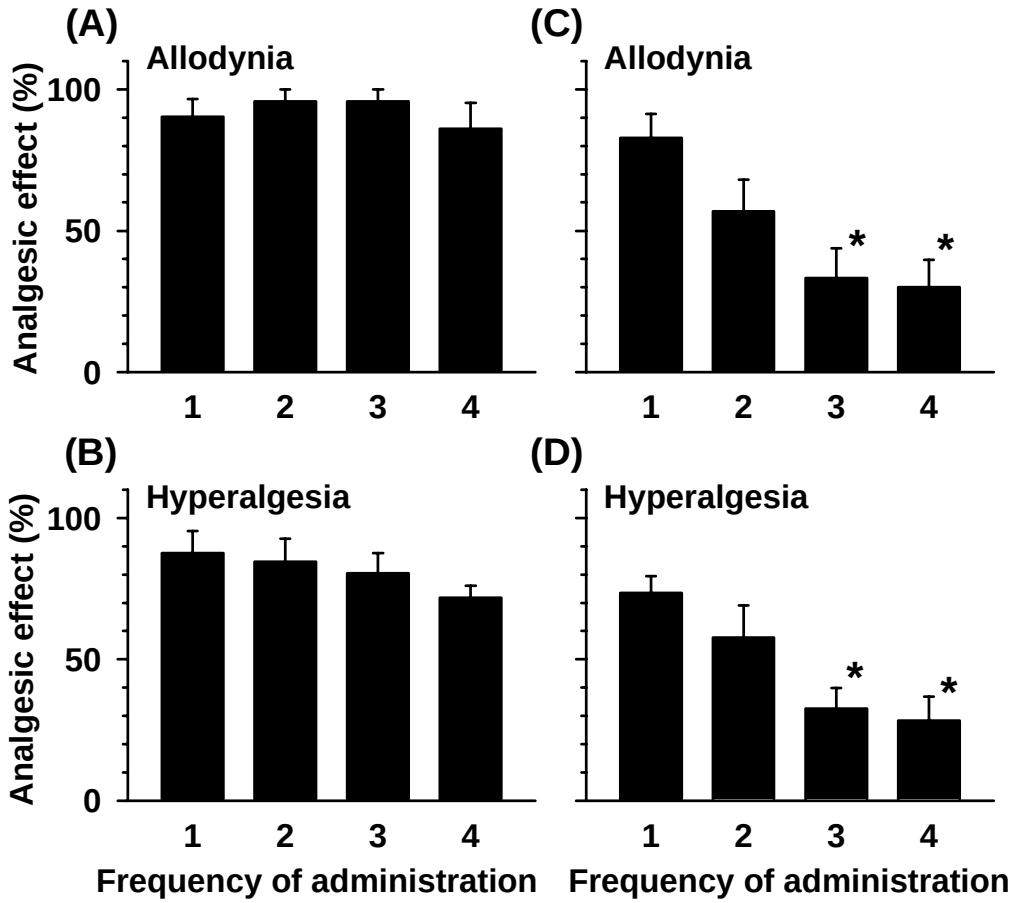
Fig. 5 (Takasaki et al.)



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