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Short communication

The novel κ-opioid receptor agonist TRK-820 has no affect on the development of antinociceptive tolerance to morphine in mice

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Abstract

The effects of the novel κ-opioid receptor agonist 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[*N*-methyl-*trans*-3-(3-furyl)acrylamido]morphinan hydrochloride (TRK-820) on the development of antinociceptive tolerance to morphine were investigated in mice and compared with those of *trans*-3,4-dichloro-*N*-(2-(1-pyrrolidinyl)-cyclohexyl) benzenacetamide methane sulfonate hydrochloride (U-50,488H), a well-defined exogenous κ-opioid receptor agonist. Morphine (1.25–20 mg/kg, s.c.) produced a dose-related antinociceptive effect in the 51°C warm-plate test. Daily treatment with morphine (10 mg/kg, s.c.) resulted in the development of antinociceptive tolerance. The development of antinociceptive tolerance to morphine was dose-dependently suppressed by the co-administration of U-50,488H (1–10 mg/kg, s.c.) with morphine, but not TRK-820 (0.003–0.03 mg/kg, s.c.). These results suggest that TRK-820-sensitive κ-opioid receptor subtypes may not be involved in modulating the development of antinociceptive tolerance to morphine.

Keywords: Morphine; TRK-820; U-50,488H; Antinociceptive tolerance; (Mouse)

1. Introduction

Morphine is an important drug in the clinical treatment of severe pain. However, tolerance develops with chronic use of morphine, resulting in a decrease in effectiveness over time. Opioid receptors are now classified into μ-, δ- and κ-opioid receptors (Knapp et al., 1995; Dhawan et al., 1996), and antinociceptive tolerance to morphine appears to be mediated primarily through μ-opioid receptors (Jiang et al., 1995). Previously, several investigators have reported that dynorphins, endogenous κ-opioid receptor agonists, and *trans*-3,4-dichloro-*N*-(2-(1-pyrrolidinyl)-cyclohexyl) benzenacetamide methane sulfonate hydrochloride (U-50,488H), a well-defined exogenous κ-opioid receptor agonist, can suppress the development of antinociceptive tolerance to morphine (Yamamoto et al., 1988; Takemori et al., 1992). These results suggest that κ-opioid receptors may play an inhibitory role in the development of antinociceptive tolerance to morphine.

Recently, Nagase et al. (1998) synthesized a novel κ-opioid receptor agonist, 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[*N*-methyl-*trans*-3-(3-furyl)acrylamido]morphinan hydrochloride (TRK-820), which has a unique structure that is different from those of well-defined benzeneacetamide compounds such as U-50,488H. The high potency and selectivity of TRK-820 to κ-opioid receptors were characterized in guinea pig ileum and mouse vas deferens preparations, while it seems that TRK-820 has an affinity for κ-opioid receptor subtypes other than U-50,488H-sensitive sites (Nagase et al., 1998). This implication is supported by additional in vivo experiments. In antinociceptive studies including acetic acid writhing, tail flick and paw pressure tests, several κ_1-opioid receptor agonists, such as U-50,488H and 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-phenyl-2-(1-pyrrolidinyl)ethyl]-acetamide (ICI-199441) and TRK-820, were all produced a

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potent antinociceptive effects, which was inhibited by the selective κ-opioid receptor antagonist nor-binaltorphimine (Endoh et al., 1999, 2000). In adjuvant-induced hyperalgesic model, the potency of antinociceptive effect of TRK-820 in arthritic group was nearly equal to that in normal group, while a selective κ_1-opioid receptor agonist ICI-199441 showed five-fold less potent antinociceptive effect (Endoh et al., 2000). It has also been reported that the ratio of the sedative ED_{50} value to the antinociceptive ED_{50} value was much greater for TRK-820 than those for a prototype of κ_1-opioid receptor agonist U-50,488H and ICI-199441 (Endoh et al., 1999). More recently, we have demonstrated that TRK-820 displays a lack of cross-tolerance to the U-50,488H-induced antinociception in mice (unpublished data). Although TRK-820 possesses the κ-opioid receptor agonistic property, these findings provide evidence for the different pharmacological profile of TRK-820 from κ_1-opioid receptor agonists. This contention is supported by another finding using conditioned place preference paradigm that TRK-820 produces neither significant aversive nor rewarding effects (Nagase et al., 1998), whereas U-50,488H reveals strong aversive effect (Funada et al., 1993). Recent physiological and binding evidence has indicated the existence of κ-opioid receptor subtypes, i.e. κ_1-, κ_2- and κ_3-opioid receptors (Zukin et al., 1988; Clark et al., 1989; Meng et al., 1993, Pan et al., 1995). Considering these behavioral data, we propose here that TRK-820 may produce its pharmacological effects via κ_1-independent κ-opioid receptor subtypes such as κ_2 and/or κ_3. Therefore, TRK-820 might be a useful tool for understanding the roles of κ-opioid receptor subtypes. In the present study, we examined the roles of κ-opioid receptor subtypes in the development of antinociceptive tolerance to morphine in mice using TRK-820 and U-50,488H.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

2.1. Animals

Male ddY mice (Tokyo Animal Laboratories, Tokyo, Japan) weighing 25–30 g were housed at a room temperature of 22 ± 1°C with a 12-h light–dark cycle (light on 0800–2000 h). Food and water were available ad libitum.

2.2. Antinociceptive assay

A 51°C warm-plate was used as the nociceptive stimulus, and the latency to paw-tap, paw-lick or an attempt to escape by jumping was considered the endpoint. Prior to administration, control latencies were determined. To prevent tissue damage, mice that showed no response within 60 s (cut-off time) were removed from the warm-plate and assigned a score of 60 s. The percent antinociception was calculated as %antinociception = 100 × (test latency-control latency)/(60 s-control latency). The antinociceptive effect of acute treatment with morphine (1.25–20 mg/kg, s.c.) was determined 30 min after drug injection. Chronic morphine (10 mg/kg, s.c.) treatment was performed between 1300 and 1400 h once daily for 6 consecutive days; the antinociceptive effect was measured before and after drug injection as above. TRK-820 (0.003–0.03 mg/kg, s.c.) or U-50,488H (1–10 mg/kg, s.c.) was co-administered with morphine.

2.3. Drugs

The drugs used in the present study were morphine hydrochloride (Sankyo, Tokyo, Japan), TRK-820 and U-50,488H. TRK-820 and U-50,488H were synthesized by us. All drugs were dissolved in saline and injected in a volume of 10 ml/kg.

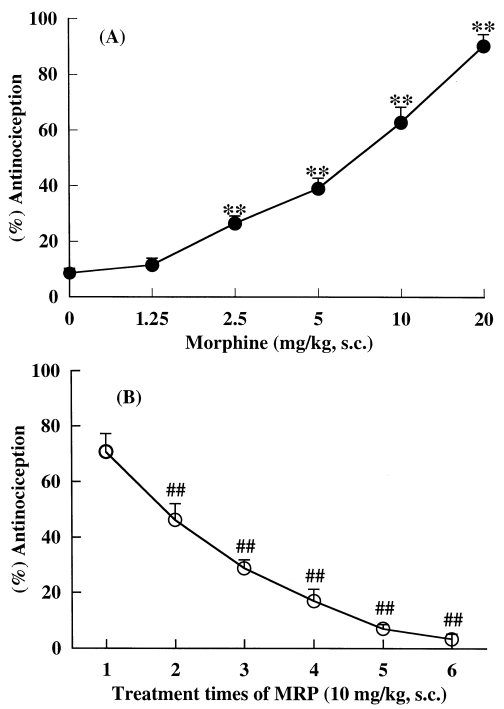

Fig. 1. Morphine-induced antinociception in the 51°C warm-plate test (A) and the development of tolerance to morphine antinociception (B). (A) Morphine (MRP: 0–20 mg/kg, s.c.) was acutely treated to mice 30 min prior to the measurement of antinociceptive activity. Each column represents the mean with S.E.M. of 10 mice. $^{**}P < 0.01$ vs. MRP (0 mg/kg, s.c.)-treated group. (B) Chronic treatment with morphine (MRP: 10 mg/kg, s.c.) was performed once daily for 6 consecutive days; the antinociceptive effect was measured before and after the drug injection every day. Each point represents the mean with S.E.M. of 10 mice. $\#\#P < 0.01$ vs. the first treatment with morphine.

2.4. Statistical analysis

The data are presented as the mean ± S.E.M. A one-way repeated measures analysis of variance (ANOVA) followed by Dunnett's test was used for the statistical evaluation ($P < 0.05$ and 0.01).

3. Results

The antinociceptive effects of various doses of morphine and the development of antinociceptive tolerance to morphine are shown in Fig. 1. Morphine (1.25–20 mg/kg, s.c.) produced a dose-related antinociceptive effect in the 51°C warm-plate test ($F(5,54) = 77.150$, $P < 0.01$) (Fig. 1A). The ED_{50} value (95% confidence limit) for morphine given s.c. was 6.57 (1.55–27.91) mg/kg. When 10 mg/kg of morphine was administered once a day for 6 consecutive days, its antinociceptive effect diminished depending on the length of treatment ($F(5,54) = 36.103$, $P < 0.01$). The antinociceptive effect produced by the second injection of morphine was significantly different from that by the first injection ($P < 0.01$). After the sixth injection of morphine, the antinociceptive effect had completely disappeared (Fig. 1B).

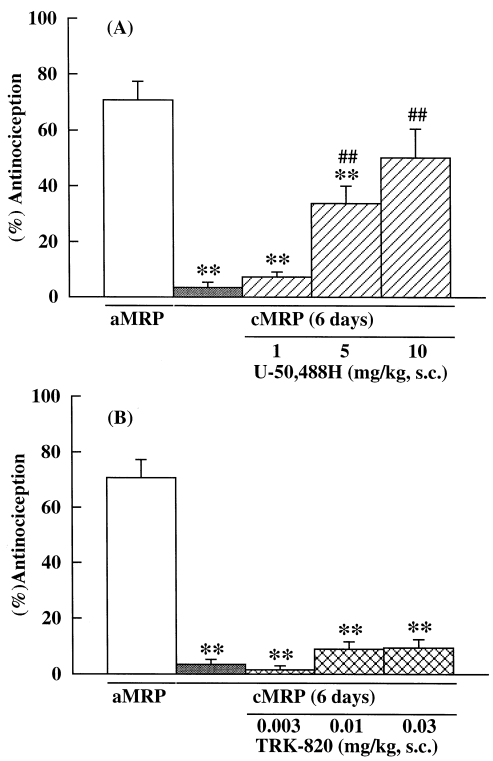

Fig. 2. Effects of U-50,488H (A) and TRK-820 (B) on the development of antinociceptive tolerance to morphine. Chronic treatment with morphine (cMRP: 10 mg/kg, s.c.) was performed once daily for 6 consecutive days; the antinociceptive effect was measured after the last treatment with morphine. U-50,488H (1–10 mg/kg, s.c.) or TRK-820 (0.003–0.03 mg/kg, s.c.) was co-administered with morphine. Each column represents the mean with S.E.M. of 10 mice. $^{**}P < 0.01$ vs. acute morphine (aMRP) group. $\#\#P < 0.01$ vs. chronic morphine (cMRP) alone group.

The effects of κ-opioid receptor agonists on the development of antinociceptive tolerance to morphine are shown in Fig. 2. Daily treatment with 10 mg/kg morphine (once daily for 6 consecutive days) resulted in the disappearance of morphine-induced antinociception, indicating the development of antinociceptive tolerance. Co-administration of U-50,488H (1–10 mg/kg, s.c.) with morphine dose-dependently suppressed the development of antinociceptive tolerance to morphine (Fig. 2A). In contrast, TRK-820 (0.003–0.03 mg/kg, s.c.) did not have any remarkable effects (Fig. 2B).

4. Discussion

In the present study, morphine produced dose-related antinociceptive effects in the 51°C warm-plate test. Moreover, chronic treatment with morphine (10 mg/kg, s.c.) once a day for 6 consecutive days resulted in a time-dependent decrease in the antinociceptive activity of morphine. The antinociceptive effect of morphine disappeared after the sixth injection, indicating the development of complete antinociceptive tolerance to morphine. Under this condition, we examined the effect of the novel κ-opioid receptor agonist TRK-820 on the antinociceptive tolerance produced by six treatments with morphine and compared with those of U-50,488H, a well-defined exogenous κ-opioid receptor agonist.

U-50,488H administered concurrently with morphine once a day for 6 consecutive days dose-dependently blocked the development of tolerance to the antinociceptive effect of morphine. Similar suppressive effects of κ-opioid receptor agonists have been shown in rats (Yamamoto et al., 1988), mice (Takahashi et al., 1991) and guinea pigs (Tao et al., 1994). We also previously reported that U-50,488H and nor-binaltorphimine, a selective κ-opioid receptor antagonist, inhibited and potentiated the development of antinociceptive tolerance to morphine, respectively (Suzuki et al., 1990, 1992). The results of the present study are in good agreement with these previous findings, confirming that κ-opioid receptors play an inhibitory role in modulating the development of antinociceptive tolerance to morphine.

In contrast to U-50,488H, the novel κ-opioid receptor agonist TRK-820 did not suppress the development of antinociceptive tolerance to morphine. Although the reason for this difference in the effects of U-50,488H and TRK-820 is not yet clear, one possibility is the differential selectivity of each compound to κ-opioid receptor subtypes. It is now known that κ-opioid receptors can be classified into at least three subtypes, i.e. κ_1-, κ_2- and κ_3-opioid receptor subtypes (Zukin et al., 1988; Clark et al., 1989; Meng et al., 1993, Pan et al., 1995). Dynorphins

bind all κ-opioid receptor subtypes, whereas U-50,488H has a high affinity for κ_1-opioid receptors (Clark et al., 1989). In contrast, Nagase et al. (1998) previously indicated the possibility that although TRK-820 exhibited high potency and high selectivity for κ-opioid receptors, the affinity of this compound for κ-opioid receptor subtypes may be different from that of U-50,488H. Moreover, recent findings by Seki et al. (1999) supported this notion, in that TRK-820 as well as naloxone benzoylhydrazone, which has been called an agonist for κ_3-opioid receptor subtypes (Gistrak et al., 1989; Price et al., 1989), significantly antagonized the nociceptin-mediated inhibition of cAMP accumulation in CHO cells expressing nociceptin receptors. It has been suggested that κ_3-opioid receptor subtypes appear to be closely related to the nociceptin receptors at the molecular level and the two receptors might result from alternative splicing of the same gene (Pasternak and Standifer, 1996; Wollemann, 1996). These reports imply that TRK-820 might have an affinity for κ_3-opioid receptor subtypes. Together with our present findings, these results suggest that U-50,488H-sensitive binding sites, i.e. κ_1-opioid receptor subtypes, rather than TRK-820-sensitive binding sites may play an inhibitory role in modulating the development of antinociceptive tolerance to morphine. However, the possibility that U-50,488H and TRK-820 differentially affect the various opioid tolerance suppression system, such as arginine vasopressin (Su et al., 1998), *N*-methyl-D-aspartate (Su et al., 1998) or cholecystokinin (Mitchell et al., 2000) system, also should not be excluded. Further investigation in relation to such other possible mechanisms would be necessary for better understanding of the relationship between morphine antinociceptive tolerance and κ-opioid receptors.

In conclusion, the present study demonstrated that coadministration of U-50,488H with morphine but not TRK-820 suppressed the development of antinociceptive tolerance to morphine, indicating the important role of κ_1-opioid receptor subtypes. The present results may constitute new important information for understanding the role of κ-opioid receptors in modulating the development of antinociceptive tolerance to morphine.

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