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Characterization of the antinociceptive effects of TRK-820 in the rat

Takashi Endoh [a,*], Atsushi Tajima [a], Tomohiko Suzuki [a], Junzo Kamei [b], Tsutomu Suzuki [c], Minoru Narita [c,d], Leon Tseng [d], Hiroshi Nagase [a]

[a] *Basic Research Laboratories, Toray Industries, 1111, Tebiro Kamakura, Kanagawa 248-8555, Japan*
[b] *Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan*
[c] *Department of Toxicology, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan*
[d] *Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA*

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Abstract

We have already reported that TRK-820, (−)-17-cyclopropylmethyl-3,14*b*-dihydroxy-4,5*a*-epoxy-6*b*-[*N*-methyl-*trans*-3-(3-furyl)acrylamido]morphinan hydrochloride, a new selective κ-opioid receptor agonist, has affinity for κ-subtype opioid receptors other than the κ_1-opioid receptor. It would be of interest to examine whether the different κ-opioid receptor subtype properties of TRK-820 participate in its antinociceptive action in the inflamed paw test and the formalin test. TRK-820 produced a potent antinociceptive effect, which was inhibited by the selective κ-opioid receptor antagonist nor-binaltorphimine, but not by the μ-opioid receptor antagonist naloxone in the mechanical paw pressure test. TRK-820 also produced a potent antinociceptive effect in rats with adjuvant-induced arthritis. TRK-820 and morphine, a prototype μ-opioid receptor agonist, were equally effective in inhibiting the nociceptive responses in the arthritic rats and in the normal rats, while ICI-199441, 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-phenyl-2-(1-pyrrolidinyl)ethyl]-acetamide, a κ-opioid receptor agonist, was about 5-fold less potent in the arthritic rats than in the normal rats. In the formalin test TRK-820 had a very similar antinociceptive potency to that of ICI-199441, unlike in the arthritic rats in which TRK-820 was 2.5 times more potent than ICI-199441. It is concluded that TRK-820 produced a potent antinociceptive action via the stimulation of κ-opioid receptors in rats. TRK-820 has a unique antinociceptive profile different from that of the other κ-opioid receptor agonists such as ICI-199441 in arthritic rats.

Keywords: κ-Opioid receptor agonist; Antinociceptive action; TRK-820; κ-Opioid receptor subtype

1. Introduction

TRK-820, (−)-17-cyclopropylmethyl-3,14*b*-dihydroxy-4,5*a*-epoxy-6*b*-[*N*-methyl-*trans*-3-(3-furyl) acrylamido] morphinan hydrochloride, is a selective κ-opioid receptor agonist which was designed and synthesized based on the ''message-address'' concept (Nagase et al., 1998) by removing ''accessory site'' from nor-binaltorphimine (Portoghese et al., 1987). In in vitro experiments using mouse vas deferens and guinea pig ileum, TRK-820 was shown to possess selective κ-opioid receptor agonistic effects (Nagase et al., 1998). We have previously demonstrated in the mouse that TRK-820 possesses a potent antinociceptive effect which is selectively mediated by the stimulation of κ-opioid receptors without any appreciable effects on μ- and δ-opioid receptors (Endoh et al., 1999). However, TRK-820 did not produce any significant place aversion or place preference using an unbiased place preference conditioning procedure in rats. This effect of TRK-820 is different from that of other κ_1-opioid receptor agonists such as U-50488H, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide, ICI-199441, 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-phenyl-2-(1-pyrrolidinyl)ethyl]-acetamide, CI-977, (5*R*)-(5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-4-benzofuranacetamide, which produce place aversion (Endoh et al., 1997), suggesting that TRK-820 produces its pharmacological effects by stimulating subtypes of κ-opioid receptors different from κ_1-opioid receptors.

TRK-820 has been administered intravenously in initial studies in humans. Single, intravenous doses of TRK-820 up to 30 μg were well tolerated by healthy volunteers,

* Corresponding author. Tel.: +81-467-32-2111; fax: +81-467-32-2127.
E-mail address: takashi_endo@nts.toray.co.jp (T. Endoh)

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whereas a dose of 40 μg TRK-820 was associated with moderate behavioral/psychological side effects, but not with psychotomimetic activity (Toray, unpublished data). So TRK-820 was again shown to be different from other κ_1 opioid receptor agonists, such as the arylacetamide spiradoline, which cause dysphoria and psychotomimetic reactions (Peters and Gaylor, 1989; Dionne et al., 1991). TRK-820 has been developed as an analgesic for moderate and severe pain and other indications.

Ligand-receptor binding studies suggested the possible existence of several subtypes of the κ-opioid receptor in the brain. The κ_1-opioid receptor binding sites were shown to have a very high selectivity and affinity for arylacetamide-like agonist such as U69593, (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzeneacetamide, and κ-opioid receptor antagonist nor-binaltorphimine. κ_2-Opioid receptor binding sites were defined as being U-69593-insensitive. In addition, κ_3-opioid receptors can be distinguished from other receptor subtypes by their unique selectivity (Clark et al., 1989; Brooks et al., 1996).

GR89696, 4-[(3,4-dichlorophenyl)acetyl]-3-(1-pyrrolidinylmethyl)-1-piperazinecarboxylic acid methyl ester, inhibited hyperalgesia and allodynia in the rat injured limb, and these actions were mediated through κ_2-opioid receptors (Eliav et al., 1999). U-69593, a κ_1-opioid agonist, did not significantly influence any assay except mechano-allodynia in rats with neuritis; however, U-69593 was only partially effective in reversing the allodynia. The present experiments were designed to examine whether another κ-site different from the κ_1-site participates in the antinociceptive action of TRK-820 in the injured paw of arthritic rats and in the formalin test. Both the mechanically induced paw pressure test and inflammatory pain models such as adjuvant-induced hyperalgesic test (Colpaert, 1987; Millan et al., 1987) and formalin test (Wheeler-Aceto and Cowan, 1991; Tjolsen et al., 1992) were used to characterize the antinociceptive properties of TRK-820 and to compare it with other κ-opioid receptor agonists.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River Japan, or Japan SLC, Japan) about 7–8 weeks of age were used. They were housed three per cage with food and water freely available until the test. A 12-h light/12-h dark daily cycle was maintained.

2.2. Paw pressure test

Mechanical nociceptive thresholds were measured using an analgesimeter (Ugo Basile, Italy) (Leighton et al., 1987). The animal was gently restrained and incremental pressure was applied via a wedge-shaped blunt piston on the ventral surface of the right hindpaw. Pressure was applied to the right hindpaw and increased at a rate of 32 g/s until withdrawal of the paw was observed. A cut-off pressure of 500 g was used to avoid tissue damage. Prior to drug administration, the pressure required to elicit paw withdrawal (i.e., paw pressure threshold) was determined three times with a 30–60 min interval occurring between measurements. The mean of the latter two threshold values was used as the predrug threshold of each rat. The test compounds were then subcutaneously (s.c.) or intramuscularly (i.m.) administered and the antinociceptive activity for each compound was measured 30 min later.

Antagonism against naloxone and nor-binaltorphimine: The antinociceptive test with or without opioid receptor antagonists was performed 30 min after the i.m. administration of TRK-820, morphine or ICI-199441. Naloxone at a dose of 0.1 mg/kg s.c. was co-administered with each agonist. Nor-binaltorphimine at a dose of 5 mg/kg was administered s.c. approximately 24 h before the injection of each agonist. These doses of the two antagonists showed a selective antagonistic action, that is, 0.1 mg/kg s.c. of naloxone antagonized the analgesia induced by morphine but not that induced by U-50488H, a κ-opioid receptor agonist, whereas 5 mg/kg s.c. of nor-binaltorphimine antagonized U-50488H analgesia but not morphine analgesia (Endoh et al., 1992).

2.3. Adjuvant-induced hyperalgesic model

A suspension (0.1 ml) containing *Mycobacterium butyricum* in paraffin oil (5 mg/ml) was inoculated intradermally in the footpad of the right hindpaw of male Wistar rats (Charles River Japan), 7 weeks old, under ether anesthesia on day 0 in order to induce arthritis (Kayser and Guilbaud, 1981). The arthritic rats were tested on day 22. The age-matched rats (10 weeks old rats) inoculated with paraffin oil only were used as controls.

2.4. Formalin test

Each animal was put into the observation chamber after administration of test compound to become adapted to the chamber until formalin injection. Fifteen minutes after test agent administration, 50 μl of 5% formalin in saline was administered s.c. into the left hind paw after the animal was removed from the chamber. Each rat was then returned to the chamber, and its licking and biting responses of the injected paw, which were defined as nociceptive responses, were recorded every 5 min for 45 min.

2.5. Drugs

The following drugs were used in this study: TRK-820, morphine hydrochloride (Takeda Japan), pentazocine (Pentagen Injection® 30 mg/ml, Sankyo, Japan), κ-opioid

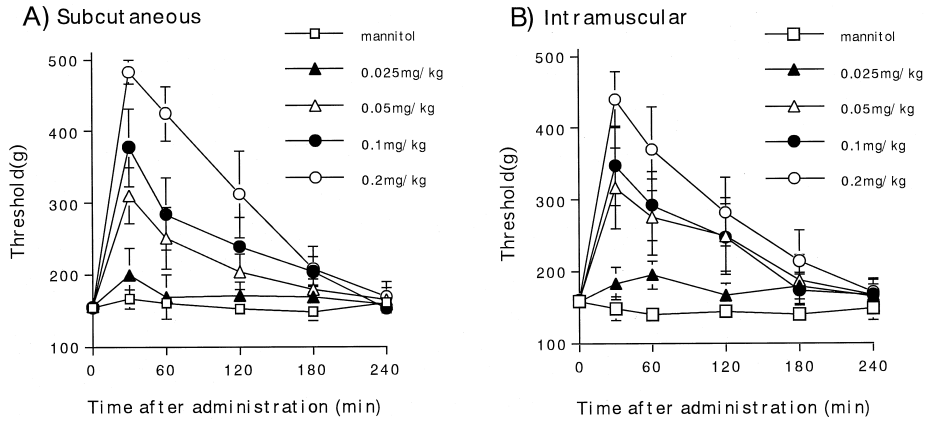

Fig. 1. Duration of antinociceptive effects of 5% mannitol and TRK-820 after s.c. (A) and i.m. (B) administration in the paw pressure test with normal rats. Each value represents the mean ± S.E.M.; $n = 6$ per point. □: 5% mannitol, ▲: TRK-820 0.025 mg/kg s.c. or i.m., △: 0.05 mg/kg s.c. or i.m., ●: 0.1 mg/kg s.c. or i.m. ○: 0.2 mg/kg s.c. or i.m.

receptor agonists such as U-50488H (Von Voigtlander et al., 1983), ICI-199441 (Costello et al., 1988), CI-977 (Hunter et al., 1990) and nor-binaltorphimine (Portoghese et al., 1987) (synthesized in Toray Research Center, Japan), and naloxone hydrochloride (RBI). TRK-820 was dissolved in 5% (w/v) D-mannitol (Towa Chemical, Japan) solution. Morphine, U-50488H, ICI-199441, pentazocine, nor-binaltorphimine and naloxone were dissolved in distilled water (Otsuka, Japan). CI-977 was dissolved in 10% (v/v) dimethylsulfoxide (Kokusan Chemical, Japan).

Test compounds were administered s.c. or i.m. in a volume of 1 ml/kg body weight 30 min before each test. All doses refer to the salt form of the drug.

2.6. Statistical analysis

In the case of the formalin test, the antinociceptive activity of each drug was defined as the percentage of inhibition of the mean duration (s) of the licking/biting response during the 45-min measurement period in the vehicle-injected group. In case of the paw pressure test (both normal and adjuvant-induced hyperalgesic rats), the individual threshold was converted to percentage of analgesia according to the following formula : % analgesia = $(T_1 - T_0)/(T_2 - T_0) \times 100$, where T_0 is predrug threshold, T_1 is the threshold after dosing, and T_2 is the cut-off (500 g) (Nakazawa et al., 1990). All data represent the mean % analgesia ± S.E.M. The dose that produced 50% analgesia was taken as the antinociceptive ED_{50} value for each agonist and was calculated from the log-dose vs. % analgesia data by linear regression techniques (Finney, 1964). Differences between pre-drug and post-drug were determined by Dunnett's multiple range test (JMP version 3.1, SAS Institute)

3. Results

3.1. Antinociceptive response to TRK-820 in the mechanically induced paw pressure test

TRK-820 at doses from 0.025 to 0.2 mg/kg given s.c. or i.m. caused dose-dependent increases in the nociceptive threshold of paw pressure responses. The antinociceptive effects reached a peak 30 min after injection, gradually declined and returned to the preinjection level 180 min after the injection (Fig. 1). Similarly, the other opioid agonists, U-50488H, ICI-199441, CI-977, morphine and pentazocine, also produced antinociception in a dose-dependent manner (data not shown). TRK-820 was found to be 160-, 2-, 20- and 70-fold more potent than U-50488H, CI-977, morphine and pentazocine, respectively, and was equipotent to ICI-199441(Table 1).

S.c. pretreatment with nor-binaltorphimine 5 mg/kg attenuated the antinociception induced by TRK-820 and the dose–response curve for TRK-820 was shifted to the right by 3-fold. However, pretreatment with naloxone 0.1

Table 1
Antinociceptive effects of various opioid drugs in the rat paw pressure test. The antinociceptive ED_{50} value of each drug was calculated from the data obtained 30 min after s.c. and i.m. administration. For the ED_{50} determination, four doses per drug and six rats per dose were used

Drugs	Antinociceptive ED_{50} value (95% confidence limits)	
	s.c. mg/kg	i.m. mg/kg
TRK-820	0.064 (0.047–0.085)	0.075 (0.051–0.11)
U-50488H	11.0 (7.3–17.0)	n.t.
ICI-199441	0.074 (0.054–0.10)	0.054 (0.030–0.098)
CI-977	0.15 (0.10–0.21)	n.t.
Morphine	1.3 (0.96–1.7)	1.6 (1.2–2.0)
Pentazocine	5.0 (3.8–6.7)	3.2 (2.5–4.1)

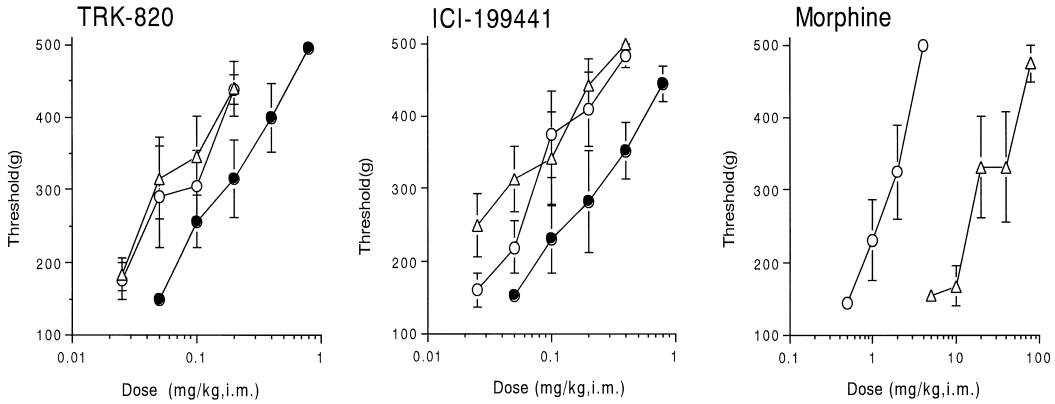

Fig. 2. Effects of nor-BNI (5 mg/kg s.c.) and naloxone (0.1 mg/kg s.c.) on the antinociceptive action induced by TRK-820, ICI-199441 and morphine in the paw pressure test with normal rats. Each value represents the mean ± S.E.M.; $n = 6$ per point. Agonist alone: ○, +nor-BNI: ●, +naloxone: △.

mg/kg s.c. did not affect the antinociception induced by i.m. administration of TRK-820. Similar results were obtained with ICI-199441; the antinociception induced by ICI-199441 was significantly inhibited by the s.c. pretreatment with nor-binaltorphimine (5 mg/kg), but not by naloxone (0.1 mg/kg). The antinociception induced by morphine was significantly attenuated by the s.c. pretreatment with naloxone (0.1 mg/kg) (Fig. 2).

3.2. Antinociceptive response to TRK-820 in the adjuvant-induced hyperalgesic test

S.c. injection of Freund's adjuvant significantly lowered the nociceptive threshold in the mechanically induced paw pressure test (the mean predrug threshold (mean ± S.E.M.) : 95.0 ± 2.20 g (arthritic, $n = 114$) vs. 153.2 ± 3.19 g (aged-matched control, $n = 90$), $P < 0.01$).

Fig. 3 illustrates the dose-related increases in paw pressure threshold observed at 30 min after i.m. administration of TRK-820, ICI-199441 and morphine in the adjuvant-arthritic rats and the age-matched normal rats. TRK-820, ICI-199441 and morphine produced potent antinociceptive activity in both the arthritic and the normal rats. TRK-820 and morphine appeared to be equally effective in inhibiting the nociceptive response in the arthritic rats and in the normal rats (the ratio of ED_{50} values in arthritic/ED_{50} values in normal rats; morphine = 1.0, TRK-820 = 1.7). However, ICI-199441 was about 5.1-fold less potent in inhibiting the nociceptive response in the arthritic rats (ED_{50} values = 0.24 mg/kg, i.m.) than in the normal rats (ED_{50} values = 0.047 mg/kg s.c.) (Table 2, Fig. 3). Furthermore, in the arthritic rats ICI-199441 was shown to be at the limit of the dose–response curve of the antinociceptive action at doses of 0.1 and 0.2 mg/kg, whereas TRK-820 as well as morphine did not show the ceiling antinociceptive action.

3.3. Antinociceptive response to TRK-820 in the formalin test

As shown in Fig. 4, the s.c. injection of 5% formalin caused an acute nociceptive response with a peak response

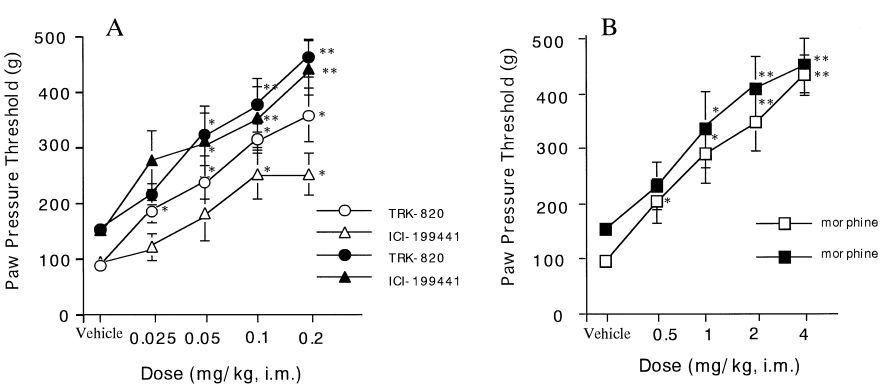

Fig. 3. Dose–response curves for TRK-820 (A), ICI-199441 (A) and morphine (B) in the paw pressure tests with age-matched normal rats (closed symbols) or adjuvant-induced arthritic rats (open symbols). Each value represents the mean ± S.E.M.; $n = 6–8$ per point. Post-drug threshold was significantly greater than pre-drug threshold; $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 2
Antinociceptive effects in age-matched normal and adjuvant-arthritic rats in the paw pressure test. The antinociceptive ED_{50} value of each drug was calculated from the data obtained 30 min after i.m. administration. The antinociceptive tests were performed 22 days after adjuvant inoculation. The age-matched rats (10-week-old rats) injected with paraffin oil only were used as normal animals. For the ED_{50} determination, four doses per drug and six to eight rats per dose were used. ( ): 95% confidence limits

Drugs	Antinociceptive ED_{50} value (mg/kg i.m.)		
	Normal	Arthritis	ED_{50} in arthritis/ED_{50} in normal
TRK-820	0.055 (0.038–0.081)	0.095 (0.063–0.14)	1.7
ICI-199441	0.047 (0.020–0.11)	0.24 (0.12–0.46)	5.1
Morphine	1.1 (0.67–1.9)	1.1 (0.75–1.7)	1.0

at 5 min after the injection and lasted about 10 min (first phase). Subsequently, this response disappeared and recurred 5–10 min later (second phase). The peak period of the second phase lasted 20–30 min.

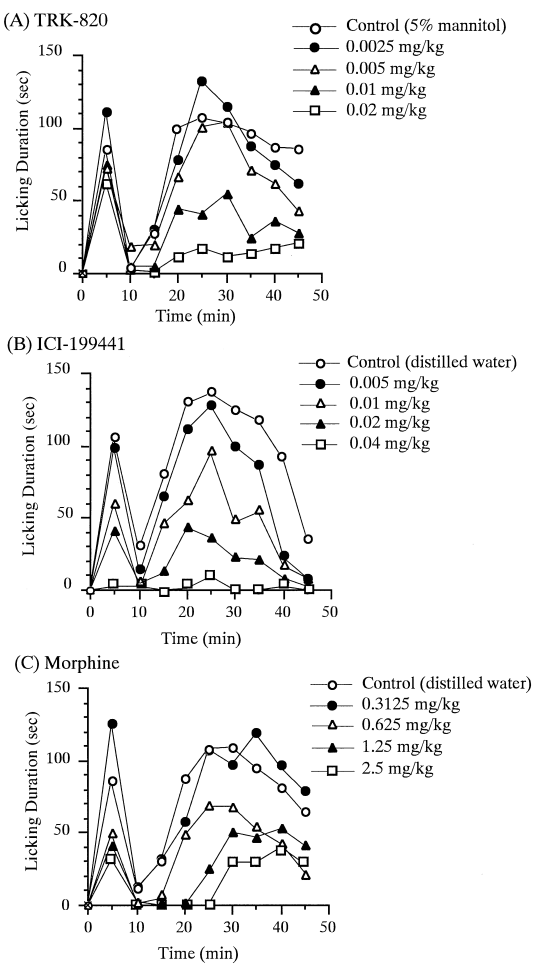

Fig. 4. Time-effect curves of s.c. TRK-820 (A), ICI-199441 (B) and morphine (C) in the rat formalin test. All agents were administered 15 min before the formalin injection. The data represent the mean (5–8 rats per point) duration of the licking/biting response per 5 min.

Table 3
Antinociceptive ED_{50} value of TRK-820, ICI-199441 and morphine in the formalin test in rats. Drugs were administered s.c. 15 min before the formalin injection. The antinociceptive ED_{50} value of each drug was defined as the dose producing a 50% reduction of the mean duration (s) of licking/biting response during the 10- to 45-min measurement period in each vehicle-injected group. Each value represents ED_{50} value with 95% confidence limits in parentheses. For the ED_{50} determination, four doses per drug and five to eight rats per dose were used

Drugs	Antinociceptive ED_{50} value (mg/kg s.c.) 15 min
TRK-820	0.0096 (0.0083–0.0112)
ICI-199441	0.0095 (0.0082–0.0109)
Morphine	0.975 (0.688–1.38)

TRK-820 at doses from 0.0025 to 0.02 mg/kg given s.c. 15 min prior to the formalin injection inhibited markedly the second phase of the formalin nociceptive response in a dose-dependent manner (Fig. 4A). However, the same doses of TRK-820 inhibited only slightly the first phase of the formalin response. Similarly, the other κ-agonist, ICI-199441, also markedly inhibited the second phase of the formalin response in a dose-dependent manner (Fig. 4B). Morphine dose dependently inhibited both phases of the formalin-induced biphasic nociceptive response. Morphine, at the dose of 2.5 mg/kg, produced marked antinociception (Fig. 4C).

In the formalin test, the antinociceptive potencies of TRK-820 and ICI-199441 were almost equal (ED_{50} values; TRK-820 = 0.0096 mg/kg s.c. vs. ICI-199441 = 0.0095 mg/kg s.c.), unlike in the arthritic rats, in which TRK-820 was 2.5-fold more potent than ICI-199441 in producing the antinociceptive action (ED_{50} values; TRK-820 = 0.095 mg/kg s.c. vs. ICI-199441 = 0.24 mg/kg s.c.) (Table 3).

4. Discussion

The results of the present studies carried out with rats are consistent with those for mice, that is, TRK-820 produced potent antinociceptive effects which were more potent than those of morphine and the other κ-opioid receptor agonists U-50488H, PD-117302, (±)-*trans*-*N*-methyl-*N*[2-(1-pyrrolidinyl)-cyclohexyl] benzo[*b*] thiophene-4-acetamide, and pentazocine, and the antinociceptive effect was selectively mediated by the stimulation of κ-opioid receptors without any appreciable effects on μ- and δ-opioid receptors (Endoh et al., 1999).

S.c. injection of TRK-820 was as effective as i.m. administration for producing antinociception in the mechanically induced paw pressure test with normal rats. There were no differences in the absorption and distribution of TRK-820 after i.m. or s.c. administration in rats, that is, the plasma level of unchanged TRK-820 when administered i.m. or s.c. reached a peak at 9–12 min, although the pharmacological effects reached a peak about 30 min later (Toray unpublished data). In the present

experiment, there were no differences in the potency and time course of the antinociceptive action between i.m. TRK-820 and s.c.TRK-820 in the paw pressure test. The pharmacological potency of the other tested opioid receptor agonist was the same for both routes of administration. We considered that the results after i.m. and s.c. administration were similar to each other.

As in mice, in rats the antinociceptive effects induced by TRK-820 are mediated by the stimulation of κ-opioid receptors. This is supported by the finding that the antinociceptive effects produced by TRK-820 were blocked by nor-binaltorphimine (5 mg/kg s.c.), but not by naloxone (0.1 mg/kg s.c.) in the paw pressure test with rats. This is consistent with the results of a previous study with mice and the acetic acid-induced writhing test (Endoh et al., 1999) in which the effects of TRK-820 were also blocked by nor-naltorphimine, but not naloxone or naltorindole.

In our experimental protocol, pressure was increased until withdrawal of the paw was observed and/or vocalization was elicited. Withdrawal of the paw was observed before vocalization was elicited. In the present studies withdrawal of the paw occurred at a pressure of 95.0 ± 2.20 g, whereas in normal rats (aged-matched control) paw withdrawal occurred at 153.2 ± 3.19 g. Similar results have been obtained by other researchers (Kayser and Guilbaud, 1990) with paw withdrawal occurring at 65–85 g in arthritic rats but at 135–210 g in normal rats. The paw withdrawal threshold is a spinally coordinated motor reflex and also modulated by supraspinally integrated mechanisms (Vycklicky, 1984). Rats with adjuvant-induced arthritis have been shown to represent an appropriate model for the evaluation of 'clinical' pain (Colpaert, 1987) and it has been reported that Freund's adjuvant treatment induces a variety of behaviors, including body weight loss and a reduction in food and water intake (Millan et al., 1987). In the present studies, adjuvant-arthritic rats displayed pronounced decreases in the threshold (g) for noxious paw pressure. This hyper-responsiveness to a noxious stimulus is a characteristic of arthritic animals (Millan et al., 1987). TRK-820 given i.m. produced potent antinociceptive activity in arthritic rats. The potency of the antinociceptive effects of TRK-820 in the arthritic rats was nearly equal to that in the age-matched normal rats, while ICI-199441, a κ_1-opioid receptor agonist, showed an approximately 5-fold less potent antinociceptive effect in the arthritic rats than in the age-matched normal rats. The findings suggest that TRK-820 stimulate subtypes of κ-opioid receptors other than κ_1-opioid receptors that are stimulated by ICI-199441. To support this possibility, others have reported that the pharmacological effects of κ-opioid receptor agonists are enhanced (Neil et al., 1986; Shippenberg et al., 1988; Stein et al., 1989) and/or decreased (Shippenberg et al., 1988) in adjuvant-arthritic rats compared with those in normal rats. GR89696 and bremazocine, κ-opioid receptor agonists different from prototype κ_1-opioid receptor agonists, inhibit hyperalgesia to heat in inflamed paws but not in non-injured paws (Eliav et al., 1999). Furthermore, μ- and δ-selective opioid receptor agonists inhibit the response of both the arthritic and the non-inflamed paws to heat, while a κ_1-selective opioid receptor agonist has no effect on the non-inflamed paw (Ho et al., 1997). It is most likely that TRK-820 produced its antinociceptive effect in the inflamed model by the stimulation of κ-opioid receptors other than κ_1-opioid receptors. In our ligand binding study using guinea pig cerebellar membranes, Scatchard analysis of [^{3}H]-TRK-820 and [^{3}H]-U-69593 binding indicated that the B_{max} value for [^{3}H]-TRK-820 (the number of [^{3}H]-TRK-820 binding sites) computed from the Scatchard plot was significantly greater than that for [^{3}H]-U-69593 (the number of [^{3}H]-U-69593 binding sites). Furthermore, under μ- and δ-opioid receptor suppressing conditions in the presence of 100 nM [D-Ala^2, MePhe^4, Gly-ol^5] enkephalin (DAMGO) (μ-opioid receptor ligand) and 200 nM [D-Pen^2, D-Pen^5]enkephalin (DPDPE) (δ-opioid receptor ligand), the number of [^{3}H]-TRK-820 binding components and the binding affinity did not change (Endoh et al., in preparation). It has been reported that guinea pig cerebellum contains predominantly κ-opioid receptors (Robson et al., 1984). In addition, only the κ_1 site has been reported to be labeled with [^{3}H]-U-69593 (Nock et al., 1988; Wollemann et al., 1992). TRK-820 also exhibited a high affinity for the κ_1-selective ligand. Taken together, we again consider that TRK-820 showed an affinity for both the κ_1-opioid receptor subtype and another κ-site different from the κ_1-opioid receptor subtype. More studies are needed to confirm this possibility.

Another complicating factor in the analysis of data obtained from inflamed and non-inflamed paw is that the baseline had shifted, which makes the comparison of dose–response curves and ED_{50} values under these circumstances more difficult. The enhanced antihyperalgesic effects of μ-opioid receptor agonists in arthritic rats has been reported. This effect has been demonstrated following systemic (Neil et al., 1986; Stein et al., 1988) or peripheral (Millan et al., 1988) injection. In the present study, the antinociceptive effects of morphine were not enhanced in arthritic rats, based on the antinociceptive ED_{50} values and the dose–response curve of morphine in the paw pressure threshold test for the inflamed paw, in which the curve was shifted to the right compared to that for the age-matched normal rats.

Biphasic nociceptive responses following the formalin injection were observed. The first phase (early phase) is recognized as being the result of the direct stimulation of the nerve endings by the stimulus and the second phase (late phase) is due to subsequent inflammation and to the hypersensitization of dorsal horn neurons (Wheeler-Aceto and Cowan, 1991; Tjolsen et al., 1992). The formalin pain model is a very useful method for characterizing analgesics. TRK-820 given s.c. inhibited markedly the second

phase nociceptive response, but was less effective in inhibiting the first phase nociceptive response. The other κ-opioid receptor agonist ICI-199441 also inhibited the first phase response less than the second phase response. Morphine inhibited almost equally well both phases. In the formalin test the antinociceptive effect of TRK-820 (ED_{50} value: 0.0096 mg/kg s.c.) was equivalent to that of ICI-199441 (ED_{50} value: 0.0095 mg/kg s.c.). In addition, the sensitivity of formalin algesia to TRK-820 and ICI-199441 was 10 times (ED_{50} value of TRK-820: 0.0096 mg/kg s.c. vs. 0.095 mg/kg, i.m.) and 25 times (ED_{50} value of ICI-199441: 0.0095 mg/kg s.c. vs. 0.24 mg/kg i.m.) higher than that of arthritic analgesia, respectively. The potency of the antinociceptive effects of morphine was nearly equal in the formalin test (ED_{50} value: 0.975 mg/kg s.c.) and adjuvant-induced hyperalgesic model test (ED_{50} value: 1.0 mg/kg s.c.). Furthermore, in the adjuvant-induced hyperalgesic model, TRK-820 was 2.5-fold more potent than ICI-199441, unlike in the paw pressure test with normal rats and in the formalin test, in which the antinociceptive potencies of TRK-820 and ICI-199441 were almost equal. Therefore, these three compounds, μ-opioid receptor agonist, κ_1-opioid receptor agonist and κ-subtype opioid receptor agonist different from an κ_1-opioid receptor agonist, differ with respect to their mode of action in formalin hyperalgesia and adjuvant-induced hyperalgesia, respectively.

In conclusion, the present study has shown that TRK-820, a selective κ-opioid receptor agonist, dose dependently produced a potent antinociceptive effect in normal rats in the paw pressure test through κ-opioid receptors and a potent antinociceptive effect against tonic-chemical/inflammatory pain. TRK-820 was shown to have a unique antinociceptive profile different from that of the κ_1-opioid receptor agonist ICI-199441 in the adjuvant-induced hyperalgesic model.

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