TRK-820, a Selective κ -Opioid Agonist, Produces Potent Antinociception in Cynomolgus Monkeys

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ABSTRACT—TRK-820 ((-)-17-cyclopropylmethyl-3,14b-dihydroxy-4,5a-epoxy-6b-[*N*-methyl-*trans*-3-(3-furyl)acrylamide]morphinan hydrochloride) has been shown to be a potent opioid κ -receptor agonist with pharmacological properties different from those produced by κ_1 -opioid receptor agonists in rodents. To ascertain whether or not these properties of TRK-820 would be extended to primates, the antinociceptive effect of TRK-820 was evaluated in cynomolgus monkeys by the hot-water tail-withdrawal procedure. TRK-820 given intramuscularly (i.m.) produced a potent antinociceptive effect that was 295- and 495-fold more potent than morphine with the 50°C and 55°C hot-water tests, respectively, and 40-fold more potent than U-50,488H and 1,000-fold more potent than pentazocine in the 50°C hot-water test. The duration of antinociceptive effects of TRK-820 treatment (0.01 and 0.03 mg/kg, i.m.) lasted more than 6 h, which was much longer than those of U-50,488H. The antinociception produced by the higher dose (0.03 mg/kg, i.m.) of TRK-820 was not inhibited by nor-binaltorphimine (3.2 and 10 mg/kg, s.c.) or by naloxone (0.1 mg/kg, s.c.), although the antinociception induced by a lower dose of TRK-820 (0.01 mg/kg, i.m.) was inhibited by nor-binaltorphimine (10 mg/kg, s.c.). The same doses of nor-binaltorphimine and naloxone effectively inhibited the antinociception induced by the higher doses of U-50,488H (1.0 mg/kg, i.m.) and morphine (10 mg/kg, i.m.), respectively. These results indicate that the antinociception induced by TRK-820 is less sensitive to nor-binaltorphimine and suggest that it is mediated by the stimulation of a subtype of κ -opioid receptor different from the κ_1 -opioid receptor in cynomolgus monkeys.

Keywords: κ -Opioid agonist, Antinociceptive effect, TRK-820, Cynomolgus monkey, κ -Opioid receptor subtype

Systematic efforts in searching for compounds which selectively stimulate κ -opioid receptors resulted in the development of the compound TRK-820, (-)-17-cyclopropylmethyl-3,14b-dihydroxy-4,5a-epoxy-6b-[*N*-methyl-*trans*-3-(3-furyl)acrylamide]morphinan hydrochloride. It has been demonstrated previously that TRK-820 possesses a highly selective κ -opioid receptor agonistic activity in bioassays with isolated guinea pig ileum and mouse vas deferens preparations (1). TRK-820 is also a selective and centrally acting κ -opioid agonist with antinociceptive and sedative activity in rodents (2). This is supported by the finding that

the antinociception induced by TRK-820 is selectively inhibited by the pretreatment with the κ -opioid receptor antagonist nor-binaltorphimine, but not by the μ -opioid receptor antagonist naloxone and δ -opioid receptor antagonist naltrindole. Unlike the other κ -opioid agonist pentazocine, which blocks μ -opioid receptors, TRK-820 has little or no μ -opioid receptor antagonistic properties. In behavioral studies, TRK-820 does not produce any significant place aversion or place preference using an unbiased place preference conditioning procedure in mice (3), although other κ -opioid receptor agonists such as U-50,488H, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidiny)-cyclohexyl]-benzeneacetamide, and E-2078, a stable dynorphin analog, produced significant place aversion (4). It has been suggested that TRK-820 produces its pharmacological effects by stimulating subtypes of κ -opioid receptors different

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from other κ -opioid receptors.

TRK-820 has been developed as an analgesic for moderate and severe pain and other indications. Clinical studies of behavioral effects of TRK-820 in humans have been performed. Single intramuscular injections of TRK-820 up to 30 μ g are well tolerated by healthy volunteers, whereas a dose of 40 μ g TRK-820 produces moderate behavioral/psychological side effects, but not psychotomimetic activity (Toray Industries, Inc., unpublished data). Thus, TRK-820 was again shown to be different from other κ -opioid receptor agonists, such as the arylacetamide spiradoline, which cause dysphoria and psychotomimetic reactions (5, 6).

In the present study, in order to ascertain whether or not these properties of TRK-820 would be extended to primates, we investigated the antinociceptive and other behavioral activities of TRK-820 and compared them with those of U-50,488H, morphine and pentazocine by using hot-water-induced tail-withdrawal tests in cynomolgus monkeys.

MATERIALS AND METHODS

Animals

Eleven adult male cynomolgus monkeys (Nihon SLC, Sizuoka) weighing between 3.0 and 8.0 kg were used for the tests. The monkeys were individually housed, fed monkey chow biscuits and given water daily except during testing. They were maintained in a room with a 12-h light / 12-h dark cycle. The monkeys were used repeatedly for the study at the intervals of 6 days or more.

Antinociceptive testing procedure

A modification of the hot-water-induced tail-withdrawal procedure described by Dykstra and Woods (7) and Dykstra et al. (8) was used. Antinociceptive activity was assessed with hot water (50°C and 55°C) as the nociceptive stimulus. On the test day, monkeys were placed in primate chairs and allowed to adapt to the test environment. The hairs of the lower portion of the tail were shaved. The tail-withdrawal response was induced by immersing monkey's tail (approximately 10 to 15 cm) in a thermos of hot water maintained at 50°C or 55°C (TAITEC, Saitama). The latency for monkeys to remove their tail from hot water was used as the measure of the antinociception. The control tail-withdrawal latencies were measured three times with 20-min intervals. The mean value of the 2nd and 3rd measurements was used as the pre-drug control latency. The tail-withdrawal latencies were then measured 0.5, 1, 2, 4 and 6 h after drug or vehicle injection. A cut-off time of 20 s was used to prevent tissue damage. Warm water (40°C) was used to ensure that the monkeys did not develop a conditioned avoidance response to the hot water stimulus. It was found that all monkeys did not respond to 40°C water immersion for more than 20 s.

Antagonistic actions of nor-binaltorphimine and naloxone

In experiments evaluating the antagonistic effects of nor-binaltorphimine and naloxone on the TRK-820-, U-50,488H- and morphine-induced analgesia, 50°C water was used as the nociceptive stimulus. Only the monkeys that had received TRK-820 alone, U-50,488H alone or morphine alone in the antinociceptive experiments using 50°C water were used in this experiment on antagonism. Pre-drug latency values were determined in the same manner as the antinociceptive testing procedure above. In case of nor-binaltorphimine antagonism, 3.2 or 10 mg/kg of nor-binaltorphimine was administered subcutaneously (s.c.), and then the antinociceptive effects produced by TRK-820, U-50,488H or morphine were measured every 7 days for 3 weeks after nor-binaltorphimine as reported (9). In the case of naloxone antagonism, TRK-820 or morphine was administered intramuscularly (i.m.) after determination of the pre-drug latency values. Forty-five minutes after administration of TRK-820 or morphine, naloxone at a dose of 0.1 or 1 mg/kg was administered by s.c. injection. The antagonism by naloxone against the antinociceptive activities was evaluated at 0.5, 1, 2, 4 and 6 h post-TRK-820 or -morphine (Note: The 30 min value represents analgesic effects of TRK-820 alone or morphine alone).

Data analyses

Individual tail-withdrawal latencies were converted to % analgesia according to the following formula: % analgesia = $(T_1 - T_0) / (T_2 - T_0) \times 100$, where T_0 is pre-latency, T_1 is the latency after dosing, and T_2 is cut-off latency (20 s). All data represent the mean % analgesia $\pm$ S.E.M. The dose that produced 50% analgesia was taken as the ED₅₀ values for each agonist, as calculated from the log-dose vs % analgesia data using linear regression techniques (10). In the case of nor-binaltorphimine antagonism, the area under the curve (AUC_{0-6h}) of % analgesia vs time (0–6 h) was calculated. Statistical significance was determined by analysis of variance followed by the Dunnett's test with JMP software (SAS Institute, Cary, NY, USA); $P < 0.05$ was considered significant.

Drugs

The following drugs were used in this study: TRK-820 (Lot No. TN-101), morphine hydrochloride (Takeda, Osaka); U-50,488H hydrochloride, a κ -opioid receptor agonist (11) (synthesized at the Toray Research Center, Inc., Kamakura); pentazocine hydrochloride (Sankyo, Tokyo); sodium pentobarbital (Tokyo Chemical Industry, Tokyo); nor-binaltorphimine (12) and naloxone hydrochloride (RBI, Natick, MA, USA). All compounds were dissolved in saline (Otsuka, Tokushima). Saline was used as the vehicle control. All test agents except antagonists were administered by intramuscular injections, and nor-binaltorphimine and naloxone were

subcutaneously administered. The volume of injections was 0.2 ml/kg body weight for all agents, except for pentazocine that was administered in a volume of 0.1 ml/kg (the dose of 3 mg/kg) and 0.33 ml/kg (the dose of 10 mg/kg). The antinociceptive test agents and doses were randomly assigned.

RESULTS

General behavior observation

TRK-820 at doses of 0.003, 0.01 and 0.03 mg/kg, given i.m. (4 animals per group), produced dose-related muscular weakness and sedation such as drowsiness, ptosis and lack of responsiveness to weak external stimuli (e.g., the sound of hand clapping or blowing the breath over the face of cynomolgus monkeys). TRK-820 as well as U-50,488H produced muscular weakness but not muscular relaxation, and intense stimuli such as pinching the skin of the limbs easily brought recovery of the muscular tension. These sedative behaviors lasted for 6 h in the group of monkeys that received 0.03 mg/kg of TRK-820. A lower dose of TRK-820 (0.001 mg/kg) produced little or no sedative behaviors. Similarly, U-50,488H at doses 0.1, 0.3 and 1 mg/kg, given i.m., produced dose-related sedative behaviors. However, the duration of the sedative behaviors produced by U-50,488H was shorter than that of TRK-820. The sedative effects reached its peak at 0.5 h, rapidly declined and returned to the pre-injection control level in 2 h in all 4 monkeys studied. Morphine at 3 and 10 mg/kg, given i.m., produced dose-related respiratory depression, drowsiness, ptosis, mydriasis and lack of responsiveness to the external stimuli in all 4 monkeys given 3 mg/kg and in all 5 monkeys given 10 mg/kg of morphine. Furthermore, 10 mg/kg of morphine produced itching behavior (face scratching) in 2 out of 5 monkeys. These sedative behaviors, respiratory depression and mydriasis lasted for 6 h in the group of monkeys injected with higher doses of morphine (10 mg/kg, i.m.). Pentazocine at a dose of 3 mg/kg, given i.m. produced sedative behaviors such as drowsiness, ptosis and lack of responsiveness to the external stimuli and these sedative behaviors dissipated in 2 h.

Inhibition of the tail-withdrawal response by TRK-820, morphine, U-50,488H and pentazocine

All monkeys tested withdrew their tails from the 50°C and 55°C hot water in 2.47 ± 0.30 s (mean $\pm$ S.E.M., $n = 11$) and 1.83 ± 0.31 s (mean $\pm$ S.E.M., $n = 11$), respectively, before the drug injection. TRK-820, morphine and U-50,488H given i.m. inhibited dose-dependently tail-withdrawal responses to 50°C hot-water stimulus (Fig. 1: A–C). On the other hand, pentazocine at 3 mg/kg produced a slight antinociceptive effect (Fig. 1D). Ten milligram of pentazocine produced tonic-chronic convulsion in

all 3 animals tested and thus the antinociceptive test could not be performed. The antinociceptive effects of TRK-820 and morphine persisted for more than 6 h, whereas the antinociception induced by U-50,488H rapidly declined and returned to the pre-injection level in 4 h. TRK-820 was found to be 295- and 40-fold more potent than morphine and U-50,488H, respectively, in the 50°C hot-water test (Table 1). Similarly, TRK-820 inhibited dose-dependently the tail-withdrawal responses to 55°C hot-water stimulus. TRK-820 was found to be 420-fold more potent than morphine in inhibiting the responses to the 55°C hot-water stimulus (Table 1). However, the antinociceptive potencies of both TRK-820 and morphine were less in the 55°C hot-water test than in the 50°C hot-water test (Fig. 2 and Table 1).

Effects of pentobarbital on behavioral and antinociceptive responses

TRK-820, morphine or U-50,488H produced antinociception at doses that also produced sedative behaviors. To determine if this antinociceptive effect produced by TRK-820, morphine or U-50,488H was due to the sedative effects of the compound that might interfere with the tail-withdrawal responses, the effect of typical central depressant pentobarbital on the tail-withdrawal responses to 50°C and 55°C hot-water stimuli was evaluated. Pentobarbital at 10 mg/kg, given i.m., produced sedative behaviors such as sleeping and/or drowsiness, yet produced little or no antinociceptive effects with 50°C and 55°C hot-water stimuli. Pentobarbital at a higher dose of 30 mg/kg produced a state of anesthesia, yet produced only a slight antinociceptive effect (approximately 20% analgesia) (Fig. 3).

Effects of nor-binaltorphimine and naloxone on the tail-withdrawal inhibition induced by TRK-820, U-50,488H or morphine

Nor-binaltorphimine at doses of 3.2 and 10 mg/kg given s.c. alone did not affect the tail-withdrawal latencies (data not shown). The pretreatment with the same doses of nor-binaltorphimine did not block the inhibition of the tail-withdrawal response induced by higher doses of TRK-820 (0.03 mg/kg, i.m.) or morphine (10 mg/kg, i.m.), but dose-dependently blocked the tail-withdrawal inhibition induced by a higher dose of U-50,488H (1 mg/kg, i.m.) (Fig. 4: A–C). A lower dose of TRK-820 (0.01 mg/kg, i.m.) produced tail-withdrawal inhibition in 2 of 4 monkeys studied, and the tail-withdrawal inhibition by TRK-820 (0.01 mg/kg, i.m.) in these two monkeys was blocked by nor-binaltorphimine (10 mg/kg, s.c.) (Fig. 4D). Naloxone, at the dose of 0.1 mg/kg, which has been reported to selectively antagonize the μ -opioid receptor (11, 13), completely reversed the antinociceptive effect of morphine and had no effect on the tail-withdrawal inhibition induced by TRK-820

Fig. 1. The antinociceptive effects of intramuscular administration of TRK-820 (A), morphine (B), U-50,488H (C) and pentazocine (D) in the hot water (50°C)-induced tail-withdrawal test in monkeys. The monkeys were intramuscularly given opioid receptor agonists and tail-withdrawal latencies were measured 0.5, 1, 2, 4 and 6 h after the treatment. Each value represents the mean $\pm$ S.E.M. The numbers of animals are shown in parentheses.

Table 1. The antinociceptive effects of intramuscular administration of TRK-820, morphine, U-50,488H and pentazocine in the warm water-induced tail-withdrawal test in monkeys

Drugs	50°C warm water		55°C warm water	
	ED ₅₀ (mg/kg, i.m.)	Duration (h)	ED ₅₀ (mg/kg, i.m.)	Duration (h)
TRK-820 ^a	0.0078 (0.0048 – 0.013)	>6	0.012 (0.0069 – 0.022)	>6
Morphine ^a	2.3 (1.4 – 3.8)	>6	5.9 (3.5 – 9.7)	>6
U-50,488H ^b	0.31 (0.22 – 0.43)	4	n.t.	n.t.
Pentazocine	>10	n.d.	n.t.	n.t.

The ED₅₀ values of antinociception were calculated from the data obtained at ^a120 min or ^b30 min after the treatment. Each value represents the ED₅₀ with 95% confidence limits (shown in parentheses). n.d.: not determined, n.t.: not tested.

Fig. 2. The antinociceptive effects of intramuscular administration of TRK-820 (A) and morphine (B) in the hot water (55°C)-induced tail-withdrawal test in monkey. The monkeys were given intramuscularly opioid receptor agonists, and tail-withdrawal latencies were measured 0.5, 1, 2, 4 and 6 h after the treatment. Each value represents the mean $\pm$ S.E.M. The numbers of animals are shown in parentheses.

Fig. 3. The effects of intramuscular administration of pentobarbital in the hot water (50°C (A) and 55°C (B))-induced tail-withdrawal test in monkey. The monkeys were intramuscularly given pentobarbital, and tail-withdrawal latencies were measured 0.5, 1, 2, 4 and 6 h after the treatment. Each value represents the mean $\pm$ S.E.M. The number of animals are shown in parentheses.

(0.03 mg/kg, i.m.) (Fig. 5). However, a higher dose (1 mg/kg) of naloxone blocked tail-withdrawal inhibition induced by both TRK-820 (0.03 mg/kg, i.m.) as well as morphine (10 mg/kg, i.m.) (Fig. 5).

Nor-binaltorphimine at the dose of 10 mg/kg, s.c. did not antagonize the muscular weakness and sedation by a higher dose (0.03 mg/kg, i.m.) of TRK-820, but antagonized these behaviors by a lower dose (0.01 mg/kg, i.m.) of TRK-820. Concerning U-50,488H and morphine as well as TRK-820, there was a parallelism in antagonism of nor-

binaltorphimine between antinociception and muscular weakness/sedation.

DISCUSSION

We have previously demonstrated in rodents that unlike other κ -opioid agonists, such as U-50,488H, TRK-820 produces potent antinociceptive effects that are mediated by the stimulation of a type of κ -opioid receptor other than the κ_1 -opioid receptor (14). In this study, we extended our

Fig. 4. The effect of nor-binaltorphimine (Nor-BNI) on the antinociceptive effects of intramuscular administration of U-50,488H (A), morphine (B) and TRK-820 (C and D) in the hot water (50°C)-induced tail-withdrawal test in monkeys. The monkeys were subcutaneously pretreated with nor-binaltorphimine 7, 14 or 21 days before the tail-withdrawal test, and tail-withdrawal latencies were measured 0.5, 1, 2, 4 and 6 h after the opioid receptor agonists treatment. Each column represents the mean $\pm$ S.E.M. of the area under the curve of % analgesia for 6 h. In each column, the number of animals are shown in parentheses. * $P < 0.05$, when compared to saline control.

studies to evaluate the antinociceptive properties of TRK-820 in the cynomolgus monkeys. We found that TRK-820 produced potent antinociceptive effects in this animal using 50°C and 55°C hot-water tail-withdrawal tests. Based on the antinociceptive ED₅₀ values, TRK-820 was 295- and 495-fold more potent than morphine with 50°C and 55°C

hot-water tail-withdrawal tests, respectively, and 40-fold more potent than U-50,488H and over 1,000-fold more potent than pentazocine with 50°C hot-water tail-withdrawal test. In addition, TRK-820 as well as morphine produced antinociception with long duration of action. The antinociceptive effects of TRK-820 and morphine persisted for

Fig. 5. The effect of naloxone on the antinociceptive effects of intramuscular administration of TRK-820 (A) and morphine (B) in the hot water (50°C)-induced tail-withdrawal test in monkeys. The monkeys were subcutaneously pretreated with naloxone 45 min (arrow) after the intramuscular administration with opioid receptor agonists, and tail-withdrawal latencies were measured 0.5, 1, 2, 4 and 6 h after the treatment. Each value represents the mean $\pm$ S.E.M. The number of animals are shown in parentheses.

more than 6 h, whereas the effect of U-50,488H lasted 4 h.

In the mouse abdominal constriction test, the antinociceptive effect of TRK-820 (0.005–0.04 mg/kg, s.c.) reached its peak at 30 min, rapidly declined and returned to the preinjection level in 2–3 h (2). In the present study, the duration of antinociception after TRK-820 (0.01 and 0.03 mg/kg, i.m.) as well as morphine (3.0 and 10.0 mg/kg, i.m.) lasted more than 6 h. The kinetics of TRK-820 in the cynomolgus monkeys and rats after i.m. administration were determined (Y. Hirano et al., unpublished data). The plasma half-lives (about 3 h) in the cynomolgus monkeys were longer than those (0.9 h) in rats when TRK-820 was administered at an i.m. dose of 0.01 mg/kg. Likewise, much longer plasma half-lives (9.2–16.7 h) were obtained after TRK-820 administered at i.m. doses of 5–25 μ g/60 kg in a clinical phase 1 study (Y. Hirano et al., unpublished data). Therefore, longer duration of antinociception of TRK-820 in primates as compared with that in rodents may be due to longer plasma half-lives, although we can not exclude the possibility that another factor influenced the duration. Concerning morphine, the plasma half-lives varied between 1.7–3.4 h in a rhesus monkey given an i.v. dose of 2 mg/kg, and an active metabolite, morphine-6-glucuronide, appeared at the same time (15). In the cynomolgus monkeys, morphine also showed a longer duration of antinociception, as compared with that in rodents (that is, the duration of antinociception was 2–3 h in the mouse abdominal con-

striction test), and the longer duration may be due to a longer plasma half-life.

It has been previously reported that nor-binaltorphimine is a long-lasting and selective κ -opioid antagonist after central or peripheral administration in rodents (16, 17). Furthermore, the antagonistic profile of nor-binaltorphimine in rodents is similar to that in primates; that is, nor-binaltorphimine acts as a selective κ -opioid antagonist with an extremely long duration of action in the hot-water (50°C and 55°C) tail-withdrawal assay in rhesus monkeys (9). In the present studies, we demonstrated the same κ -opioid receptor blocking properties of nor-binaltorphimine in cynomolgus monkeys. Pretreatment (1 to 3 weeks before) with 3.2 and 10 mg/kg of nor-binaltorphimine inhibited dose-dependently the antinociceptive effects of the higher dose (1.0 mg/kg, s.c.) of κ -opioid agonist U-50,488H (Fig. 4).

We have previously reported that TRK-820 has unique profiles for antinociception that are different from those produced by other κ -opioid receptor agonists such as ICI-199441 in arthritic rats (14). While the antinociceptive potency of ICI-199441 is about fivefold less in arthritic rats than in the age-matched normal rats, the antinociceptive potency of TRK-820 in arthritic rats is similar to that in the age-matched normal rats. In the present study, both the higher dose of TRK-820 (0.03 mg/kg, i.m.) and U-50,488H (1.0 mg/kg, s.c.) showed maximum antinociceptive responses; that is, the peak responses of the antinociception of

TRK-820 and U-50,488H were $100 \pm 0\%$ and $96 \pm 4\%$, respectively, although the antinociception of U-50,488H was of a markedly shorter duration than that of TRK-820. In the present antagonism study using nor-binaltorphimine, the antinociceptive effects produced by a higher dose of TRK-820 (0.03 mg/kg, s.c.) was not inhibited by the pretreatment with nor-binaltorphimine (3.2 and 10 mg/kg, s.c.), although the antinociception induced by a lower dose of TRK-820 (0.01 mg/kg, s.c.) was inhibited by nor-binaltorphimine (10 mg/kg, s.c.). The same doses (3.2 and 10 mg/kg, s.c.) of nor-binaltorphimine dose-dependently inhibited the antinociception induced by the higher dose (1.0 mg/kg, s.c.) of U-50,488H. However, in the mouse abdominal constriction test, nor-binaltorphimine antagonized the antinociception induced by TRK-820 and that by U-50,488H to similar extents, and the dose-response curves of TRK-820 and U-50,488H were shifted to the right by 39-fold and 15-fold, respectively (2). In the rat mechanically induced paw pressure test, nor-binaltorphimine also antagonized the antinociception by TRK-820 and that by ICI-199441, a κ_1 -opioid agonist, to similar extents (14). Therefore, the antinociceptive action of TRK-820 was less sensitive to antagonism by nor-binaltorphimine as compared with U-50,488H in the cynomolgus monkeys, unlike in rodents.

Pretreatment with naloxone at a lower dose (0.1 mg/kg), which selectively blocks μ -opioid receptors (11, 13), completely blocked the antinociception induced by the higher dose (10 mg/kg) of morphine, but not the higher dose (0.03 mg/kg, s.c.) of TRK-820, indicating that the action of TRK-820 is not mediated by μ -opioid receptors in cynomolgus monkeys. However, naloxone is a non-selective opioid receptor antagonist and blocks κ - and other opioid receptors as well as μ -opioid receptors at high doses. We found that naloxone at 1 mg/kg blocked both TRK-820- and morphine-induced antinociceptive effects. When the pharmacological properties of TRK-820 were analyzed using Chinese hamster ovary cells expressing cloned rat κ -, μ - and δ -opioid receptors and human nociceptin receptor, TRK-820 was shown to act as a full agonist for the κ -opioid receptor, a partial agonist for the μ -opioid receptor, and a low-affinity antagonist for the nociceptin receptor (18). We can not deny the possibility that TRK-820 is relatively resistant to nor-binaltorphimine in cynomolgus monkeys because of partial μ -agonistic action. However, as mentioned above, the agonist effects of TRK-820 as well as U-50,488H are sensitive to antagonism by nor-binaltorphimine both in smooth muscle preparations *in vitro* and in analgesic tests using rodents *in vivo* (1, 2, 14). Therefore, in the present experiments using cynomolgus monkeys, TRK-820 may selectively act as a agonist on κ -opioid receptors without μ -agonistic and μ -antagonistic effects, at least within antinociceptive dose range. Taken together, our results suggest that TRK-820 stimulates κ -receptors that are different

from those stimulated by U-50,488H.

TRK-820 as well as U-50,488H produced muscular weakness and sedation but not muscular relaxation, and arousal stimuli such as pinching the skin of the limbs or shaking the limbs easily brought a recovery of the muscular tension. In the present experiments, we used pentobarbital as a negative control drug because pentobarbital also produced muscular weakness and sustained relatively weak muscular tension. Like morphine (19) or U-50,488H (20), TRK-820 produced muscular weakness and sedation at doses that produce antinociception. However, the sedative effects produced by TRK-820 and other opioids were less severe than those produced by the general central depressant pentobarbital. This is supported by the finding that pentobarbital even at high doses did not inhibit the tail-withdrawal responses to hot-water stimuli. Therefore, it is most likely that antinociception measured by the 50°C or 55°C hot-water tail-withdrawal responses is not due to the influence of the sedative effects of TRK-820 and other opioid agonists, and the inhibition of the tail-withdrawal response is thought to be the antinociceptive action.

In summary, TRK-820 produced potent and long-lasting antinociceptive effects in cynomolgus monkeys. The antinociceptive effect induced by TRK-820 may be mediated by the stimulation of a subtype of κ -opioid receptors different from the κ_1 -opioid receptor. TRK-820 also produced sedative effects, which is distinguished from the antinociceptive effects.

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