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Short communication

Supraspinal δ_1-opioid receptor-mediated antinociceptive properties of (−)-TAN-67 in diabetic mice

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Abstract

The antinociceptive potencies of the enantiomorphs of TAN-67 (2-methyl-4*a*α-(3-hydroxyphenyl)-1,2,3,4,4*a*,5,12,12*a*α-octahydro-quinolino[2,3,3,–*g*]isoquinoline), (−)-TAN-67 and (+)-TAN-67, given intracerebroventricularly (i.c.v.) on the antinociceptive response were studied in streptozotocin-induced diabetic mice using the tail-flick test. (−)-TAN-67 at doses of 3–10 μg given i.c.v. produced dose-dependent inhibition of the tail-flick response in both non-diabetic and diabetic mice. The antinociceptive effect of (−)-TAN-67 in the tail-flick test in diabetic mice was greater than that in non-diabetic mice. The antinociceptive effect of (−)-TAN-67 was not antagonized by pretreatment with either β-funaltrexamine, a selective μ-opioid receptor antagonist, or nor-binaltorphimine, a selective κ-opioid receptor antagonist. When 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist, was administered 10 min before treatment with (−)-TAN-67, the antinociceptive effect of (−)-TAN-67 was significantly antagonized. However, naltriben, a selective δ_2-opioid receptor antagonist, had no significant effect on the antinociceptive effect of (−)-TAN-67. On the other hand, in the tail-flick test, (+)-TAN-67 at doses of 3–30 μg given i.c.v. did not produce dose-dependent inhibition of the tail-flick response in either non-diabetic or diabetic mice. In conclusion, (−)-TAN-67, but not its enantiomer (+)-TAN-67, produced an antinociceptive effect through the activation of δ_1-opioid receptors.

Keywords: (−)-TAN-67; (+)-TAN-67; Antinociception; δ-Opioid receptor; 7-Benzylidenenaltrexone; Naltriben, diabetes

1. Introduction

(±)-TAN-67 (2-methyl-4*a*α-(3-hydroxyphenyl)-1,2.3,4,4*a*,5,2,12*a*α-octahydro-quinolino[2.3,3–*g*]isoquinoline) is a non-peptidic δ-opioid receptor ligand. Preliminary studies have shown that (±)-TAN-67 has a high affinity for δ-opioid receptors ($K_i = 0.7$ nM) in rat brain with a 2070-fold lower affinity at μ-opioid receptors and a 1600-fold lower affinity at κ-opioid receptors (Nagase et al., 1994). Knapp et al. (1995) also reported that (±)-TAN-67 exhibited a high binding affinity ($K_i = 0.647$ nM) at human δ-opioid receptors and high δ-opioid receptor selectivity (> 1000-fold), relative to that for μ-opioid receptors. (±)-TAN-67 is a potent δ-opioid receptor agonist with an EC_{50} value of 1.72 nM for the inhibition of the forskolin-stimulated cAMP accumulation at human δ-opioid receptors expressed by intact Chinese hamster ovary cells (Knapp et al., 1995) and an EC_{50} value of 4.4 nM for the inhibition of the contraction of the mouse deferens (Nagase et al., 1994).

However, the high potency and selectivity of this racemic mixture of TAN-67 on δ-opioid receptors observed in in vitro studies is not consistent with findings in vivo that (±)-TAN-67 produced weak, if any, antinociceptive responses. (±)-TAN-67 given i.c.v. or systemically was not active in inhibiting the tail-flick and hot-plate responses and showed a low potency in inhibiting acetic acid-induced abdominal constriction after systemic administration (Kamei et al., 1995; Suzuki et al., 1995). On the other hand, we recently reported that the antinociceptive effect of (±)-TAN-67 in inhibiting acetic acid-induced

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abdominal constriction in diabetic mice was greater than that in non-diabetic mice (Kamei et al., 1995). We previously reported that the antinociceptive effect of [D-Pen^{2,5}]enkephalin (DPDPE) administered i.c.v. was significantly greater in diabetic mice than in non-diabetic mice (Kamei et al., 1994). The antinociceptive effect of DPDPE was antagonized in both diabetic mice and non-diabetic mice following pretreatment with 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist, but not with naltriben, a selective δ_2-opioid receptor antagonist. Furthermore, there was no significant difference in the antinociceptive effect of [D-Ala^2]deltorphin II, administered i.c.v. in diabetic and non-diabetic mice. The antinociceptive effect of [D-Ala^2]deltorphin II was antagonized in both diabetic mice and non-diabetic mice following pretreatment with naltriben, but not with 7-benzylidenenaltrexone. Based on these results, we suggested that mice with diabetes are selectively hyper-responsive to supraspinal δ_1-opioid receptor-mediated antinociception (Kamei et al., 1994).

The enantiomeric forms of (±)-TAN-67 have recently been resolved. The present study was designed to characterize the antinociceptive properties of (−)-TAN-67 and (+)-TAN-67 after i.c.v. injection in mice. Furthermore, to test our hypothesis that diabetic mice are selectively hyper-responsive to supraspinal δ_1-opioid receptor-mediated antinociception, we assessed the antinociceptive effect produced by i.c.v. (−)-TAN-67 and (+)-TAN-67 in diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science Co., Tokyo, Japan), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at 22 ± 1°C with a 12-h light-dark cycle. Animals were rendered diabetic by an injection of streptozotocin (200 mg/kg, i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with the vehicle alone. The experiments were conducted 2 weeks after injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic. The experiments described here were performed in accordance with the guide for the care and use of laboratory animals as adopted by Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture, Japan.

2.2. Antinociceptive assay

The antinociceptive response was evaluated by recording the latency in the tail-flick test using radiant heat as a stimulus. The intensity of the thermal stimulus was initially adjusted so that the animal flicked its tail in 2–3 s. A cut-off latency of 15 s was used to prevent injury to the tail. Animals which did not respond within 15 s were removed and assigned a score of 15 s. The percent maximum possible effect (%MPE) was calculated for each animal using the formula: %MPE = 100 × (post-drug latency − pre-drug latency)/(15 − pre-drug latency).

2.3. Drugs

(−)- and (+)-TAN-67, β-funaltrexamine, 7-benzylidenenaltrexone, naltriben and nor-binaltorphimine were synthesized in Nagase's laboratory. All of the drugs were dissolved in saline. β-Funaltrexamine (20 mg/kg, s.c.) was injected 24 h before testing. 7-Benzylidenenaltrexone (0.3 mg/kg) and naltriben (0.3 mg/kg, s.c.) were injected 10 min before injection of TAN-67, as previously described (Kamei et al., 1994). Nor-binaltorphimine (20 mg/kg, s.c.) was injected 3 h before TAN-67 injection (Endoh et al., 1992). Intracerebroventricular (i.c.v.) administration (5 μl/mouse) was performed according to the method described by Haley and McCormick (1957) using a 50-μl Hamilton syringe.

2.4. Statistical analysis

The data are expressed as the mean with S.E. The statistical significance of differences was assessed with the Newman-Keuls test for the comparison of percent antinociception. The ED_{50} values and their 95% confidence intervals for the antinociceptive effect of TAN-67 were calculated using a linear standard curve. A level of probability of 0.05 or less was accepted as significant.

3. Results

Groups of mice were injected i.c.v. with different doses of (−)-TAN-67 (3–30 μg) and the tail-flick response was measured 30 min after the injection. I.c.v. injection of (−)-TAN-67 caused a dose-dependent increase in tail-flick inhibition in both non-diabetic and diabetic mice (Fig. 1A). The dose-response curve for (−)-TAN-67 in diabetic mice was shifted to the left of that in non-diabetic mice (Fig. 1A). The ED_{50} values (μg with 95% confidence limits) were 52.9 (12.8–219.0) and 6.2 (4.8–8.1) for non-diabetic and diabetic mice, respectively. Thus, the antinociceptive potency of (−)-TAN-67 in diabetic mice was significantly greater than that in non-diabetic mice. As shown in Fig. 1B, however, (+)-TAN-67 at doses from 3 to 30 μg given i.c.v. did not produce a dose-dependent antinociceptive effect in either diabetic or non-diabetic mice.

The effects of 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist, naltriben, a selective δ_2-opioid receptor antagonist, β-funaltrexamine, a selective μ-opioid

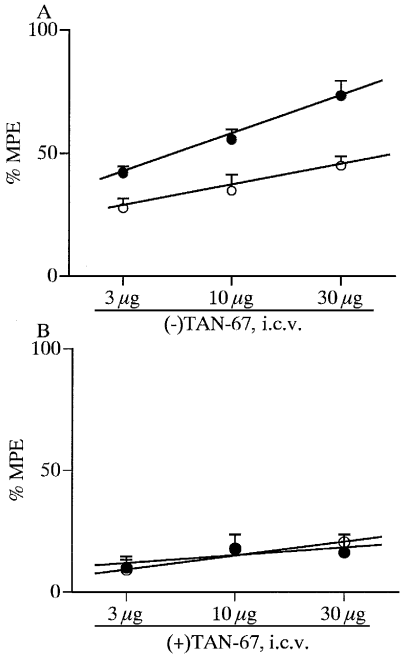

Fig. 1. Dose-response curves for the antinociceptive effect of the i.c.v. administration of (−)-TAN-67 (A) and (+)-TAN-67 (B) in tail-flick responses in both non-diabetic (open circles) and diabetic (closed circles) mice. Each point represents the mean with S.E. for eight mice in each group.

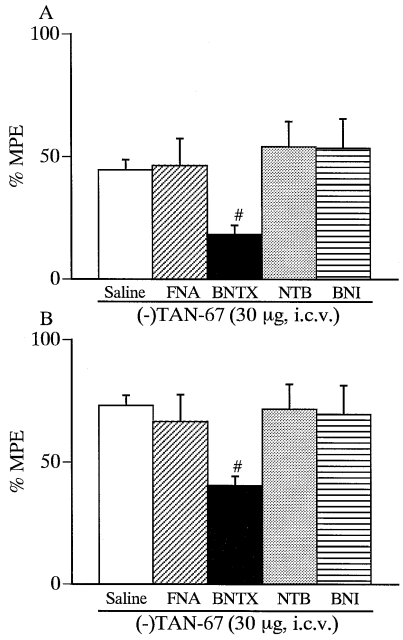

Fig. 2. Blockade of the antinociceptive effects of (−)-TAN-67 (30 μg, i.c.v.) by opioid antagonists in non-diabetic (A) and diabetic (B) mice. 7-Benzylidenenaltrexone (BNTX, 0.3 mg/kg) or naltriben (NTB, 0.3 mg/kg) was injected s.c. 10 min before administration of (−)-TAN-67. β-Funaltrexamine (FNA, 20 mg/kg) was injected s.c. 24 h before the test. Nor-binaltorphimine (BNI, 20 mg/kg, s.c.) was injected 3 h before administration of (−)-TAN-67. Each column represents the mean with S.E. for eight mice in each group. * $P < 0.05$ versus the saline-treated group.

receptor antagonist, and nor-binaltorphimine, a selective κ-opioid receptor antagonist, on the antinociceptive effect of (−)-TAN-67 are summarized in Fig. 2. The antinociceptive effect of (−)-TAN-67 was not antagonized by pretreatment with either β-funaltrexamine or nor-binaltorphimine in both diabetic and non-diabetic mice. However, when 7-benzylidenenaltrexone was administered 10 min before administration of (−)-TAN-67, the antinociceptive effect of (−)-TAN-67 was significantly antagonized in both diabetic and non-diabetic mice. In contrast, naltriben had no significant effect on the antinociceptive effect of (−)-TAN-67 in either diabetic or non-diabetic mice. The mean %MPE produced by 7-benzylidenenaltrexone, naltriben, β-funaltrexamine, and nor-binaltorphimine, by themselves, was $12.8 \pm 4.2\%$, $12.6 \pm 3.0\%$, $10.9 \pm 6.2\%$ and $15.1 \pm 6.2\%$, respectively, in non-diabetic mice, and $9.9 \pm 5.6\%$, $13.9 \pm 4.3\%$, $11.2 \pm 6.3\%$ and $9.6 \pm 5.5\%$ in diabetic mice.

4. Discussion

The present study demonstrated that i.c.v. administration of (−)-TAN-67 caused a dose-dependent increase in tail-flick inhibition in both non-diabetic and diabetic mice. I.c.v.-administered (−)-TAN-67-induced antinociception was markedly antagonized by s.c. pretreatment with 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist (Portoghese et al., 1992), but not by naltriben, a selective δ_2-opioid receptor antagonist (Sofuoglu et al., 1991), β-funaltrexamine, a selective μ-opioid receptor antagonist, or nor-binaltorphimine, a selective κ-opioid receptor antagonist, indicating that the antinociceptive effect of (−)-TAN-67 results from the activation of δ_1-opioid receptors.

The present study also demonstrated that i.c.v. administration of (−)-TAN-67 in diabetic mice produced a marked and dose-dependent antinociceptive effect in the tail-flick test. The antinociceptive effect of (−)-TAN-67 given i.c.v. in diabetic mice was also antagonized by systemic pretreatment with a δ_1-, but not with a μ-, κ-, or δ_2-opioid receptor antagonist, as in non-diabetic mice. Furthermore, the results of the present experiments clearly indicate that the antinociceptive potency of (−)-TAN-67 is significantly greater in diabetic mice than in non-diabetic mice. The results of this study agree with our previous finding that the δ_1-opioid receptor-mediated antinociceptive effect of a racemic mixture of (±)-TAN-67 is significantly greater in diabetic mice than in non-diabetic mice (Kamei et al., 1995) and support our hypothesis that diabetic mice are selectively hyperresponsive to supraspinal δ_1-opioid receptor-mediated antinociception (Kamei et al., 1994).

Recently, we demonstrated that unlike (−)-TAN-67, which produces antinociception, (+)-TAN-67 given i.t. produces hyperalgesia (Tseng et al., 1997). This conclusion is based on the finding that (+)-TAN-67 given i.t. decreased the latencies of the tail-flick response at low

doses (1.8–8.9 nmol) and produced scratching and biting at higher doses (17.9–89.4 nmol) (Tseng et al., 1997). However, in the present study, we found that (+)-TAN-67 given i.c.v. had no significant effects on the tail-flick latencies in both non-diabetic mice and diabetic mice. Furthermore, (+)-TAN-67 given i.c.v. did not produce abnormal behaviors, such as scratching or biting. Although the exact mechanism is not yet clear, it is possible that the site of action of (+)-TAN-67 for producing hyperalgesia exists in the spinal cord, but not in a supraspinal site. We previously reported that a racemic mixture of (±)-TAN-67, which binds potently to δ-opioid receptors in in vitro binding studies (Knapp et al., 1995), does not inhibit the tail-flick responses in non-diabetic mice after systemic (s.c.) injection (Kamei et al., 1995). It is possible that the weak, if any, antinociceptive response following (±)-TAN-67 given s.c. is associated with the hyperalgesic effect and the absence of an effect of (+)-TAN-67 when given i.t. or i.c.v., respectively.

In conclusion, the present results indicate that (−)-TAN-67 given i.c.v. produces greater supraspinal δ_1-opioid receptor-mediated antinociception in diabetic mice than in non-diabetic mice in the tail-flick assay.

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