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Antinociceptive effects of the selective non-peptidic δ-opioid receptor agonist TAN-67 in diabetic mice

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Abstract

The antinociceptive potencies of 2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydro-quinolino[2,3,3-*g*]isoquinoline (TAN-67), a non-peptidic δ-opioid receptor agonist, were examined using the acetic acid abdominal constriction test and the tail-flick test in diabetic mice. TAN-67, at doses of 3–100 mg/kg, i.p., produced a marked and dose-dependent inhibition of the number of acetic acid-induced abdominal constrictions in both non-diabetic and diabetic mice. The antinociceptive effect of TAN-67 in the acetic acid abdominal constriction test in diabetic mice was greater than that in non-diabetic mice. Indeed, the ED_{50} (95% confidence limits) value of TAN-67 for the inhibition of acetic acid-induced abdominal constrictions in diabetic mice (6.0 (3.5–10.5) mg/kg) was significantly lower than that in non-diabetic mice (31.4 (14.2–69.4) mg/kg). 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 (0.3 mg/kg, s.c.), 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. Furthermore, in the tail-flick test, TAN-67 at doses of 3–30 mg/kg, i.p., also produced a marked and dose-dependent antinociceptive effect in diabetic mice, but not in non-diabetic mice. In conclusion, TAN-67 produced an antinociceptive effect through the activation of δ_1-opioid receptors. Furthermore, the results of this study support our hypothesis that mice with diabetes are selectively hyperresponsive to δ_1-opioid receptor-mediated antinociception.

Keywords: TAN-67; Antinociception; δ-Opioid receptor; 7-Benzylidenenaltrexone; Naltriben diabetes

1. Introduction

Recent studies have demonstrated the existence of two δ-opioid receptor subtypes, which have been referred to as δ_1-and δ_2-opioid receptors, using selective peptidic δ-opioid receptor agonists, [D-Pen^{2,5}]enkephalin (DPDPE) and [D-Ala^2]deltorphine II (Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991a,b). We recently identified a non-peptidic selective δ-opioid receptor agonist, 2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydro-quinolino[2,3,3-*g*]isoquinoline (TAN-67, Fig. 1), that demonstrates a high degree of selectivity for the δ-opioid receptor in vitro (Nagase et al., 1994). This compound shows a high affinity for δ-opioid receptors (K_i = 1.12 nM) and a low affinity for μ-opioid receptors (K_i = 2320 nM) and κ-opioid receptors (K_i = 1790 nM) in rat brain (Nagase et al., 1994). Therefore, although its selectivity for the δ-opioid receptor subtypes is not well known, TAN-67

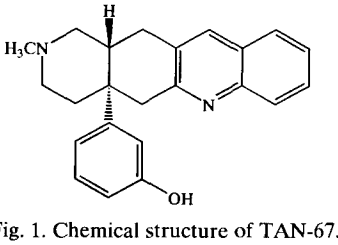

Fig. 1. Chemical structure of TAN-67.

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might be useful for elucidating the pharmacological and physiological functions of δ-opioid receptors.

We recently reported that the antinociceptive effects of μ-opioid receptor agonists, such as morphine and [D-Ala^2,*N*-MePhe^4,Gly-ol^5]enkephalin (DAMGO), in diabetic mice were markedly less than those in non-diabetic mice (Kamei et al., 1992a,c, 1994a). Therefore, we suggested that diabetic mice are selectively hyporesponsive to μ-opioid receptor-mediated antinociception (Kamei et al., 1992a,c, 1994a). On the other hand, we previously demonstrated that an endogenous δ-opioid receptor-mediated antinociceptive system is enhanced in diabetic mice, as compared to non-diabetic mice (Kamei et al., 1992a,b). Furthermore, the antinociceptive potency of DPDPE, which is reversed by 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist (Portoghese et al., 1992), was significantly greater in diabetic mice than in non-diabetic mice (Kamei et al., 1993, 1994b). However, there was no significant difference in the antinociceptive effect of [D-Ala^2]deltorphine II, which is reversed by naltriben, a selective δ_2-opioid receptor antagonist (Portoghese et al., 1991), between diabetic and non-diabetic mice (Kamei et al., 1994b). These findings suggested that mice with diabetes are selectively hyperresponsive to δ_1-opioid receptor-mediated antinociception, but are normally responsive to activation of δ_2-opioid receptor-mediated antinociception (Kamei et al., 1994b).

Thus, the first aim of the present study was to investigate the influence of 7-benzylidenenaltrexone and naltriben on TAN-67-induced antinociception to determine the role of the δ-opioid receptor subtypes in the antinociceptive effect of TAN-67. An additional aim of this study was to investigate the effect of diabetes on TAN-67 to clarify our hypothesis that diabetic mice are selectively hyperresponsive to δ_1-opioid receptor-mediated pharmacological action, but not to that mediated by δ_2-opioid receptors.

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. Studies in this report were performed in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by Hoshi University.

2.2. Antinociceptive assay

The antinociceptive effect was evaluated using the acetic acid abdominal constriction test. Each mouse was injected i.p. with 0.7% acetic acid in a volume of 10 ml/kg, 30 min after administration of the test drug. After a 10-min delay, the animals were observed for an additional 10 min, during which the number of abdominal constrictions was counted. The number of abdominal constrictions in each test period was normalized to the mean number shown by the control group. Percent antinociception was expressed as: 100 × (mean control responses − test response)/(mean control responses).

In a separate series of experiments, the antinociceptive effect was also evaluated by recording the latency in the tail-flick test using radiant heat as a stimulus. The intensity of the thermal stimulus was adjusted so that the animal flicked its tail in 2–3 s. A cut-off latency of 10 s was used to prevent injury to the tail. Animals which did not respond within 10 s were removed and assigned a score of 10 s. Percent antinociception was calculated for each animal using the formula: 100 × (post-drug latency − pre-drug latency)/(10 − pre-drug latency).

2.3. Drugs

TAN-67, β-funaltrexamine, 7-benzylidenenaltrexone, naltriben and nor-binaltorphimine were synthesized by Dr. H. Nagase (Toray Industries, Kamakura, Japan). 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., 1994b). Nor-binaltorphimine (20 mg/kg, s.c.) was injected 3 h before TAN-67 injection (Endoh et al., 1992).

2.4. Statistical analysis

The data are expressed as the means ± S.E. The statistical significance of differences was assessed with the Newman-Keuls test for the comparison of percent antinociception, and with the Mann-Whitney U-test for comparison of the number of abdominal constriction responses. The ED_{50} values, the ED_{50} ratio and their 95% confidence intervals for the antinociceptive effect TAN-67 were computed according to Litchfield and Wilcoxon (1949) using Program 47 of the Pharmaco-

logical Calculations system of Tallarida and Murray (1987). A level of probability of 0.05 or less was accepted as significant.

3. Results

3.1. Effect of TAN-67 on acetic acid-induced abdominal constriction

Intraperitoneal injection of 0.7% acetic acid induced abdominal constriction responses in both non-diabetic (39.1 ± 3.1/10 min, $n = 8$) and diabetic mice (39.0 ± 4.3/10 min, $n = 8$). There was no significant difference in the number of acetic acid-induced abdominal constrictions between non-diabetic and diabetic mice.

Intraperitoneal administration of TAN-67, at doses of 3–100 mg/kg, resulted in a marked and dose-dependent reduction in the number of acetic acid-induced abdominal constrictions in both non-diabetic and diabetic mice (Fig. 2). The dose-response curve for TAN-67 in diabetic mice was shifted to the left of that in non-diabetic mice (Fig. 3). The ED_{50} values (mg/kg with 95% confidence limits) were 31.4 (14.2–69.4) and 6.0 (3.4–10.5) for non-diabetic and diabetic mice, respectively. The ED_{50} ratio (95% confidence limits) of the antinociceptive effect of TAN-67 in diabetic mice versus that in non-diabetic mice was 5.4 (1.8–16.3).

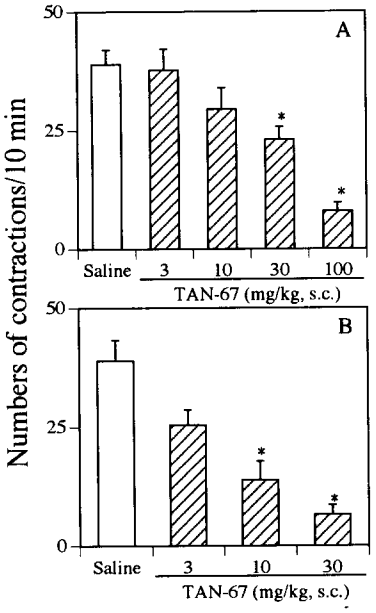

Fig. 2. Effect of TAN-67 on the number of acetic acid-induced abdominal constrictions in non-diabetic (A) and diabetic (B) mice. Each mouse received an i.p. injection of 0.7% acetic acid 30 min after the administration of TAN-67. After a 10-min delay, the animals were observed for an additional 10 min, during which the number of abdominal constrictions was counted. Each column represents the mean ± S.E. for eight mice in each group. $^*P < 0.05$ versus the respective saline-treated group.

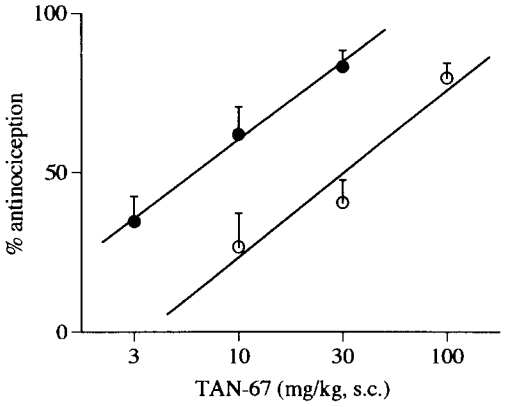

Fig. 3. Dose-response lines for the antinociceptive effect of i.p. administration of TAN-67 in the acetic acid-induced abdominal constriction assay in both non-diabetic (open circle) and diabetic (closed circle) mice. Each point represents the mean ± S.E. for eight mice in each group.

Thus, the antinociceptive potency of TAN-67 in diabetic mice was significantly greater than that in non-diabetic mice.

3.2. Effects of selective opioid-receptor antagonists on the antinociceptive effect of TAN-67

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

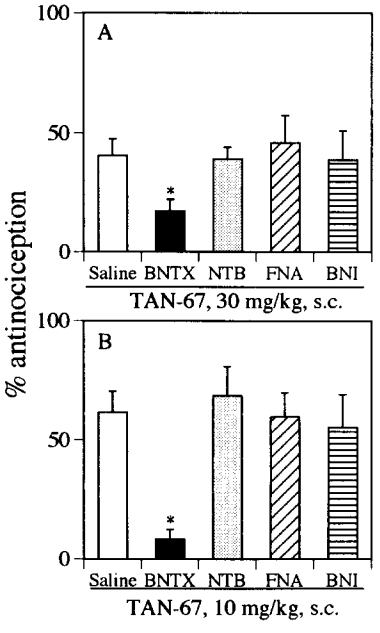

Fig. 4. Blockade of the antinociceptive effects of TAN-67 by opioid antagonists in non-diabetic (A) and diabetic (B) mice. 7-Benzylidenenaltrexone (BNTX, 0.3 mg/kg) or naltriben (NTB, 0.3 mg/kg) was injected s.c. 10 min before administration of TAN-67. β-Funaltrexamine (FNA, 20 mg/kg) was injected s.c. 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 ± S.E. for eight mice in each group. $^*P < 0.05$ versus the saline-treated group.

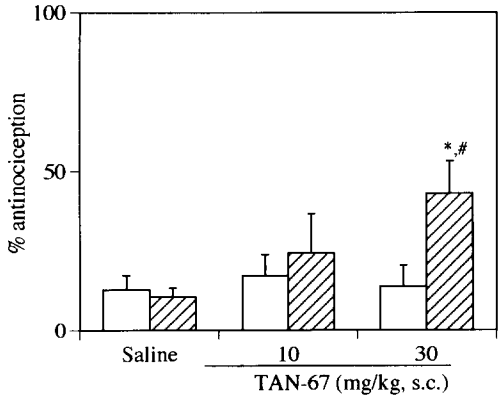

Fig. 5. Dose-response relationship of the antinociceptive effect of TAN-67 in diabetic (hatched column) and non-diabetic (open column) mice in the tail-flick assay. The antinociceptive effects of TAN-67 were measured in the tail-flick assay 30 min after injection. Each column represents the mean ± S.E. for eight mice in each group. $^{*}P < 0.05$ versus the respective saline-treated group. $^{\#}P < 0.05$ versus the values for non-diabetic mice.

opioid receptor antagonist, β-funaltrexamine, a selective μ-opioid receptor antagonist and nor-binaltorphimine, a selective κ-opioid receptor antagonist, on the antinociceptive effect of TAN-67 are summarized in Fig. 4. 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 percent antinociception produced by 7-benzylidenenaltrexone, naltriben, β-funaltrexamine, and nor-binaltorphimine, by themselves, was 13.8 ± 4.9%, 12.2 ± 5.0%, 9.6 ± 3.2% and 11.1 ± 6.2%, respectively, in non-diabetic mice, and 9.6 ± 6.5%, 10.9 ± 5.3%, 9.2 ± 5.3% and 10.9 ± 5.5% in diabetic mice.

3.3. Effects of TAN-67 on the tail-flick responses

As shown in Fig. 5, TAN-67 at doses of 3–30 mg/kg, i.p., also produced a marked and dose-dependent antinociceptive effect in diabetic mice as assessed by the tail-flick test. However, TAN-67 did not produce any significant antinociceptive effect in non-diabetic mice in this test (Fig. 5).

4. Discussion

The present study demonstrated that i.p. administration of TAN-67 reduced the number of acetic acid-induced abdominal constrictions in a dose-dependent manner in non-diabetic mice. 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 (Portoghese 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. Recently, Chang et al. (1993) have identified a novel non-peptidic δ-opioid receptor agonist, (±)-4-((α-*R**)-α-((2*S**,5*R**)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-*N*,*N*-diethylbenzamide (BW373U86), that demonstrates a selectivity for δ-opioid receptors. However, the δ-opioid receptor selectivity of BW373U86 (μ/δ dissociation constant ratio of 8.3) in rat brain using radioligand competition assays (Chang et al., 1993) was markedly less than that of TAN-67 (μ/δ dissociation constant ratio of 2070; κ/δ dissociation constant ratio of 1600) (Nagase et al., 1994). Furthermore, the antinociceptive effects of i.p. BW373U86 in an abdominal constriction assay were partially antagonized by i.c.v. naloxone, but not by i.c.v. naltrindole, a selective $\delta_{1/2}$-opioid receptor antagonist (Wild et al., 1993). In contrast, both i.t. naloxone and i.t. naltrindole antagonized the antinociceptive effect of BW373U86 (Wild et al., 1993). Therefore, Wild et al. (1993) suggested that the antinociceptive effect of BW373U86 at the spinal level appears to involve a δ-opioid receptor-mediated mechanism, whereas the supraspinal effects appear to be associated with interactions at μ-opioid receptors. The present study clearly demonstrated that the antinociceptive effect of i.p. TAN-67 was antagonized by systemic pretreatment with a δ_1-, but not with a μ, κ or δ_2-opioid receptor antagonist. It is possible that the antinociceptive effect of TAN-67 at both the supraspinal and spinal levels may be associated with interactions at δ_1-opioid receptors. These results suggest that TAN-67 is a selective δ_1-opioid receptor agonist, and may be a better tool than BW373U86 for determining the pharmacological role of δ_1-opioid receptors.

The present study also demonstrated that i.p. administration of TAN-67 in diabetic mice produced a marked and dose-dependent antinociceptive effect in the acetic acid abdominal constriction assay. The antinociceptive effect of i.p. TAN-67 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, TAN-67 also increased the tail-flick latency in a dose-dependent manner in diabetic mice, but had no significant effect on the tail-flick latency in non-diabetic mice. 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. These results suggest that diabetic mice are hyperresponsive to the δ_1-opioid receptor-mediated antinociceptive effects of TAN-67. The results of this study agree with our previous finding that the antinociceptive effect of DPDPE, a proposed δ_1-opioid receptor agonist, is significantly greater in diabetic mice than in non-diabetic mice (Kamei et al., 1994b), and support our hypothesis that diabetic mice are selectively hyperresponsive to δ_1-opioid receptor-mediated antinociception.

In conclusion, the present results indicate that TAN-67 produces greater δ_1-opioid receptor-mediated antinociception in diabetic mice than in non-diabetic mice in both the acetic acid-induced abdominal constriction assay and the tail-flick assay.

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