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Research report

The novel δ opioid receptor agonist KNT-127 produces antidepressant-like and antinociceptive effects in mice without producing convulsions

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ABSTRACT

We previously reported that the δ opioid receptor (DOP) agonists SNC80 and TAN-67 produce potent antidepressant-like and antinociceptive effects in rodents. However, SNC80 produced convulsive effects. Recently, we succeeded in synthesizing a novel DOP agonist called KNT-127. The present study examined the convulsive, antidepressant-like, and antinociceptive effects of KNT-127 in mice. In contrast to SNC80, KNT-127 produced no convulsions at doses of up to 100 mg/kg. In mice subjected to the forced swim test, a screening model for antidepressants, KNT-127 (1 mg/kg, s.c.) significantly decreased the duration of immobility and increased the duration of swimming without influencing spontaneous locomotor activity. These behavioral changes were similar to that observed for the tricyclic antidepressant imipramine (6 mg/kg). The antidepressant-like effect of KNT-127 in mice was antagonized by pretreatment with naltrindole (NTI), a selective DOP antagonist, or naltriben, a putative DOP_2 subtype antagonist. In addition, KNT-127 (3 mg/kg, s.c.) significantly reduced the number of acetic acid-induced abdominal constrictions and the duration of licking time, respectively, in mice subjected to a writhing test and a formalin test. These antinociceptive effects were antagonized by pretreatment with either NTI or 7-benzylidenenaltrexone, a putative DOP_1 subtype antagonist. We propose that KNT-127 should be considered as a candidate compound for the development of DOP-based antidepressants and/or analgesics that lack convulsive effects.

1. Introduction

The biological effects of endogenous opioid peptides are mediated through three classes of naloxone-sensitive opioid receptors: mu (μ), kappa (κ), and delta (δ). All three opioid receptors are present throughout the central and peripheral nervous systems. δ opioid receptors (DOPs) are located in the cerebral cortex, striatum, amygdala, brainstem nuclei, and spinal cord [1]. This distribution is consistent with the location of major regions involved in modulation of pain and mood [2]. The δ-opioidergic system, in particular, is recognized as a novel neurotransmitter system that may be directly involved in anxiety and depression [3–6]. On the basis of these findings, many have proposed DOPs as attractive targets for the development of novel analgesics and antidepressants [7–11].

Although only one DOP gene has been cloned thus far [12,13], pharmacological studies suggest that at least two DOP subtypes are expressed: the putative δ_1 opioid receptor subtype (DOP_1) and the putative δ_2 opioid receptor subtype (DOP_2). The putative DOP_1 subtype is preferentially activated by [D-Pen^2, D-Pen^5] enkephalin and antagonized by 7-benzylidenenaltrexone (BNTX), while the putative DOP_2 subtype is preferentially activated by [D-Ala^2, D-Glu^4] deltorphin and blocked by naltriben (NTB) [10]. Several studies on the antinociceptive actions of combined DOP subtype agonists and antagonists support these findings [14–18]. On the other hand, some of the pharmacological effects of DOP agonists may appear through partial activation of other opioid receptors [19,20] or heterodimer forms of DOP receptors [21].

A number of small-molecule DOP agonists have become available for experimental use; they comprise compounds having different structures, such as the isoquinoline derivative TAN-67 [22] and the benzhydryl piperazine derivatives SNC80 [23] and BW373U86 [24]. We previously reported that systemic adminis-

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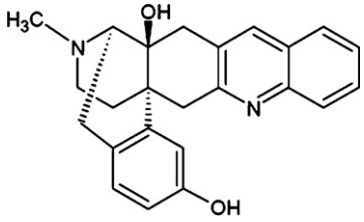

Fig. 1. Chemical structure of KNT-127.

tration of TAN-67 produces an antinociceptive effect via putative DOP_1, but not DOP_2, in the acetic acid-induced abdominal constriction (writhing) test in a mouse model of visceral pain [22,25]. In contrast to our TAN-67 results, Broom et al. [26] indicated that BW373U86 produced an antinociceptive effect via both putative DOP_1 and DOP_2 in the writhing test in mice. In addition to the antinociceptive effects of these DOP agonists, we and other investigators reported that both TAN-67 and SNC80 produce an antidepressant-like effect in rats [4,8] and mice [27,28] in the Porsolt forced swim test, which is a reliable tool for screening antidepressants [29,30]. These effects were completely blocked by naltrindole (NTI), which can antagonize both DOP_1 and DOP_2. These findings were supported by results from other studies using the forced swim test [31] and an olfactory bulbectomy model of depression [11,32]. Unfortunately, SNC80 and BW373U86 have pronounced side effects, such as causing convulsions in rodents and monkeys [33–36].

Recently, Nagase et al. [37] succeeded in synthesizing a novel DOP agonist called KNT-127 (Fig. 1), which was designed based on the structure of TAN-67 and its derivative SN-28 [38]. KNT-127 showed higher affinity for DOPs (Ki value = 0.16 nM) than TAN-67 and lower affinity for opioid μ receptors (Ki value = 21.3 nM) and opioid κ receptors (Ki value = 153 nM). Consequently, KNT-127 showed higher selectivity for DOPs than SN-28 [37]. These results indicate that KNT-127 has sufficient affinity and selectivity for DOPs and thus may be a useful tool for clarifying pharmacological effects (e.g., antinociceptive, antidepressant-like, and epileptogenic effects) mediated by DOPs. We found that intrathecal (i.t.) and subcutaneous (s.c.) injection of KNT-127-induced strong antinociceptive effects in the writhing test [37]. However, the selectivity of DOP in the KNT-127-induced antinociceptive effect was unclear. In addition, it remains unknown whether KNT-127 has any antidepressant-like and convulsive effects.

Therefore, in the present study, we examined the antidepressant-like and antinociceptive effects of KNT-127 systemically administered to mice. We also examined the convulsive effects of KNT-127 and SNC80 in mice.

2. Materials and methods

2.1. Animals

Male ICR mice (6–8 weeks of age) weighing 30–40 g were purchased from Japan SLC, Inc. and housed in standard polycarbonate mouse cages (16 cm × 22 cm × 14 cm; 4–5 mice per cage) for at least 2 weeks prior to the experimental procedures. All mice were kept under standard laboratory conditions: 12 h light/dark cycle, 21–24 °C room temperature, and free access to tap water and standard mouse diet. The study protocol was in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the ethics review committee for animal experiments in the National Institute of Neuroscience, Japan (Approval No. 2010001). All efforts were made to minimize the number of animals used and their suffering.

2.2. Apparatus and experimental procedure

2.2.1. Forced swim test

The procedure was a modification of the Porsolt et al. [29] method developed by Cryan et al. [30]. Mice were placed individually for 10 min in glass cylinders (diameter, 20 cm; height, 30 cm) that were filled with water (25 ± 1 °C) to a depth of 15 cm for each training session. Twenty-four hours after their exposure, the animals were placed in a swim apparatus for 10 min and their performance was measured the last 5 min of the test session. The behaviors measured during the test session were as follows: immobility (the mouse was judged immobile whenever it stopped all active behaviors and remained floating in the water with minimal movements, with its head just above the water); swimming (movements throughout the swim cylinder); and climbing (upward-directed movements of the forepaws along the cylinder walls). A time-sampling technique was used whereby the predominant behavior (immobility, swimming, or climbing) in each 5 s period of the 300 s test session was recorded. The water was changed and the cylinder rinsed with clean water to avoid the influence of alarm substances. The apparatus was placed in indirect light (20 lx). Measurements were observed on a monitor through a video camera in a separate area. KNT-127 or imipramine was administered 30 min before each test session. NTI, BNTX, or NTB were administered subcutaneously (s.c.) 30 min before KNT-127 administration.

2.2.2. Measurement of locomotor activity

Locomotor activity was monitored by using an activity sensor unit for mice (AS-TIME/Ver.1, O'Hara & Co. Tokyo, Japan) and by using polycarbonate cages (16 cm × 22 cm × 14 cm). Each animal was placed into the apparatus, which was located beneath indirect lighting (40 lx), as previously described [39]. Locomotor activity was measured for 30 min before injection (habituation to the apparatus) and 90 min after drug administration. Data were analyzed to determine the distance traveled per 5 min period.

2.2.3. Antinociceptive assay

An antinociceptive assay was performed using an acetic acid-abdominal constriction (writhing) test [40,20] and a formalin test [41], based on previous methods. Briefly, for the writhing test, each mouse was injected intraperitoneally (i.p.) with 0.6% acetic acid at a dose of 10 ml/kg 15 min after KNT-127 administration (s.c.). After a 10 min delay, the animals were observed for an additional 10 min, during which the number of abdominal constrictions was counted. Percent inhibition was calculated and compared with the number of writhing movements in the control group. NTI, BNTX, or NTB was administered s.c. 30 min before KNT-127 administration (s.c.). For the formalin test, each mouse was injected s.c. with 20 μl of 5% formalin (formaldehyde solution 37% [w/w] diluted in saline) into the dorsal surface of the right hind paw after KNT-127 administration. The behavioral manifestations of nociception (paw licking, shaking, or biting) were measured from 20 to 30 min after the injection of formalin. A late phase lasting from 20 to 30 min after the injection of formalin was defined as the "early inflammatory response" [41]. NTI was administered s.c. 30 min before KNT-127 administration.

2.2.4. Measurement of convulsive effects

Convulsive effects on mice were measured as described previously [34]. Mice were injected s.c. with either KNT-127 or SNC80 and immediately placed into individual glass circular cages (diameter, 10 cm; height, 15 cm) containing bedding and observed for the duration of the observation period. Mice were observed for convulsions and subsequent catalepsy-like behavior for 20 min after drug treatment. The convulsions occurred as clonic movements of the head and forepaws. The mouse was judged to exhibit post-convulsion catalepsy-like behavior if it failed to immediately remove its paws from a horizontal rod (located 5 cm from the cage floor) and if it failed to display the righting reflex. The measurement of convulsive events and duration of catalepsy-like behavior were recorded by observers. The apparatus was placed in indirect light (20 lx).

2.3. Drugs

The drugs used in the present study were KNT-127, NTI, BNTX, and NTB (synthesized by Nagase et al., Kitasato University, Tokyo, Japan) and SNC80 (synthesized by Toray Industries, Inc., Kanagawa, Japan). The doses and NTI, BNTX, and NTB administration schedules were determined according to our previous methods [15,42]. All drugs were dissolved in saline. All drugs or vehicle (saline) were administered in a volume of 0.1 ml per 10 g of body weight.

2.4. Data analysis

Data are expressed as means ± S.E.M. The statistical significance of the differences in the behavioral data was assessed by one-way analysis of variance (ANOVA), and post hoc individual group comparisons were made using the Bonferroni test (GraphPad Prism 4). *P* values less than 0.05 were considered significant.

3. Results

3.1. The effect of KNT-127 on active behaviors in a modified forced swim test

The effects of KNT-127 and imipramine on the performance of mice on the modified forced swim test are shown in Fig. 2.