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Research report

Pharmacological characterization of the ameliorating effect on short-term memory impairment and antinociceptive effect of KT-90 in mice

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Abstract

(−)-3-Acetyl-6β-acetylthio-*N*-cyclopropylmethyl-normorphine (KT-90) is a synthesized compound that binds to μ-, δ- and κ-opioid receptors in vitro. KT-90 induces analgesia in the tail-flick test and this effect is antagonized by nor-BNI, a selective κ-opioid receptor antagonist. However, lower doses of KT-90 antagonize morphine-induced analgesia. We reported that κ-opioid receptor agonists such as U-50,488H and dynorphin A (1-13), improved scopolamine-induced impairment of learning and memory in mice and/or rats. In this study, the effects of KT-90 were investigated in an acetic acid-induced writhing test and scopolamine-induced memory impairment test using spontaneous alternation performance in a Y-maze. Male ddY mice were treated with scopolamine (1.65 μmol/kg, s.c.) 30 min before the behavioral test. KT-90 (0.07–2.35 μmol/kg, s.c.) was injected 30 min before testing. In the writhing test, the antinociceptive effect of KT-90 (0.71 μmol/kg) was completely antagonized by a selective μ-opioid receptor antagonist, β-funaltrexamine (10.2 nmol/mouse, i.c.v.) and partially antagonized by nor-BNI (4.9 nmol/mouse, i.c.v.), but it was not antagonized by a selective δ-opioid receptor antagonist, naltrindole (9.1 pmol/mouse, i.c.v.). KT-90 significantly improved the impairment of spontaneous alternation induced by scopolamine. The ameliorating effect of KT-90 was not antagonized by nor-BNI, but was almost completely antagonized by a selective σ receptor antagonist, NE-100 (2.6 μmol/kg, i.p.). These results suggested that the KT-90-induced antinociceptive effect was mediated by μ- and partially by κ-opioid receptors, and the KT-90-induced improvement in scopolamine-induced impairment of spontaneous alternation was mediated mainly via σ receptors.

Keywords: KT-90; σ Receptor; κ-Opioid receptor; Spontaneous alternation; Short-term memory; Antinociceptive effect

1. Introduction

The compound (−)-3-acetyl-6β-acetylthio-*N*-cyclopropylmethyl-normorphine (KT-90) [13] binds to μ-, κ- and δ-opioid receptors with 4-, 19- and 13-fold higher affinity than morphine in rat brain membrane preparations [21]. The antinociceptive effects of KT-90 are 5–10 times as potent as those of morphine in the rat paw pressure test and in the mouse acetic acid-induced writhing test [21]. The antinociceptive effect of KT-90 is antagonized by nor-BNI, a κ-opioid receptor antagonist, but not by β-FNA, a μ-opioid receptor antagonist [23]. Katsumata et al. [14] reported that although KT-90 acted as a partial agonist of the μ-, δ- and κ-opioid receptors, because of an extremely low efficacy at the μ-opioid receptor, KT-90 antagonized the effect of morphine in the μ-, but not δ- or κ-opioid receptors at concentrations of 10 and 100 nM. As a result, a low dose of KT-90 antagonizes morphine-induced analgesia [23]. Further, KT-90 also produces conditioned place aversion [23] as does by U-50,488H [1], but KT-90 only produced conditioned place aversion at a high dose (10 mg/kg = 23.5 μmol/kg, s.c.). These findings suggest that KT-90 acts on the κ-opioid receptor agonist and

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μ-opioid receptor antagonist similar to pentazocine, while its effect on the δ-opioid receptor is unclear. Since KT-90 had stronger analgesic activity than morphine, and might not possess psychic dependence liability [23], it seems to be a potent clinical analgesic acting on κ-opioid receptors.

Previously, we reported that κ-opioid receptor agonists such as U-50,488H and dynorphin A (1-13), improved learning and memory impairment using several amnesia models in mice and rat [5,6,7,8,10]. It is well recognized that the analgesic effects of (±)-pentazocine are due to agonistic actions at κ1-opioid receptors [18]. Pentazocine is widely used clinically as an analgesic [2,18], but is less analgesic than morphine [18]. We have found that the ameliorating effect of not only (+)-pentazocine but also (−)-pentazocine on the scopolamine-induced impairment of spontaneous alternation behavior was antagonized by a σ receptor antagonist, NE-100 [9]. These findings led us to speculate that KT-90 has similar characteristics to pentazocine. If so, KT-90 may be effective against learning and memory impairments involving the cholinergic systems.

In this study, the improvement of short-term memory by KT-90 following scopolamine treatment in mice was investigated using spontaneous alternation behavior in a Y-maze. Furthermore, we re-examined the analgesic effects of KT-90 using an acetic acid-induced writhing test in mice.

2. Materials and methods

2.1. Animals

Male 7–9 weeks ddY mice (30–40 g, Japan SLC, Japan) were kept in a controlled environment, with a 12-h light/12-h dark cycle and given food and tap water ad libitum. Experimental protocols concerning the use of laboratory animals were approved by the committee of Meijo University and were performed in accordance with the guidelines of the Japanese Pharmacological Society (Folia Pharmacol. Japon, 1992, 99: 35A) and the interministerial decree of 25th May 1987 (The Ministry of Education).

2.2. Drugs

The following drugs were used: (−)-3-acetyl-6β-acetylthio-*N*-cyclopropylmethyl-normorphine (KT-90) [13]; *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylenoxy)-phenyl]-ethylamine monohydrochloride (NE-100, Taisho Pharmaceuticals Inc., Tokyo, Japan); norbinaltorphimine (nor-BNI, Research Biochemicals Inc., Natrick, MA); and scopolamine hydrobromide (scopolamine, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). Drugs were dissolved in an isotonic saline solution (Otsuka Pharmaceuticals Inc., Tokyo, Japan). Nor-BNI was administered immediately before the administration of KT-90 into the lateral ventricle (i.c.v.) of the mouse brain according to the methods of Haley and McCormick [3] at a volume of 5 μl/mouse under brief ether anesthesia. KT-90 and/or scopolamine were administered subcutaneously (s.c. around neck and abdominal areas) 30 min before and NE-100 was administered intraperitoneally (i.p.) 30 min before the behavioral experiments. Control mice received saline s.c. or i.p. at a volume of 0.1 ml/10 g body weight and/or i.c.v. at a volume of 5 μl/side in mouse brain.

2.3. Acetic acid-induced writhing test

The writhing test was conducted at 30 min after s.c. injection of the test drugs. Mice were treated with a 0.7% acetic acid solution and placed in individual Plexiglas boxes for observation. Ten minutes after the injection of acetic acid, the number of writhing responses was recorded for a 10-min period.

2.4. Spontaneous alternation behavior

Immediate working memory performance was assessed by recording spontaneous alternation behavior during a single session in a Y-maze with minor modification [5,19]. Each mouse, new to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entries into the three arms, in overlapping triplet sets. The effect was calculated as percentage alternation according to the following formula:

$$\text{Percent alternation} = \left(\frac{\text{number of alternations}}{\text{total number of arm entries}} - 2 \right) \times 100$$

The spontaneous alternation test in the Y-maze was used as a first-intent test for the anti-amnesic effects of κ_1-opioid receptor agonists and σ receptor agonists, since it is pharmacologically predictive, does not constrain the animals, and we have several data using this method accompanied with a passive avoidance test.

2.5. Data analysis

The behavioral data are expressed in terms of the mean ± S.E.M. for the writhing test, and median (vertical column) and interquartile ranges, first to third quartiles (vertical line) for the Y-maze test. Since data for the Y-maze test did not always show Gaussian distribution, we analyzed it using non-parametric type statistical methods. Thus, the significance of differences was evaluated using Student's *t*-test or a one-way analysis of variance followed by Bonferroni's test for the writhing test, and using the Mann–Whitney *U*-test or the Kruskal–Wallis test followed by Bonferroni's test for multiple comparisons in the Y-maze test. The criterion for significance was $P < 0.05$ in all statistical evaluations.

3. Results

3.1. Antinociceptive effect of KT-90 in the acetic acid-induced writhing test

KT-90 (0.07–0.71 μmol/kg, s.c.) had dose-dependent antinociceptive effects and 0.24 and 0.71 μmol/kg of KT-90 significantly decreased the number of writhing responses (Fig. 1).

The antinociceptive effect of KT-90 was antagonized by a κ-opioid receptor antagonist, nor-BNI (4.9 nmol/mouse, i.c.v.) (Fig. 2A) and a μ-opioid receptor antagonist, β-FNA (10.2 nmol/mouse, i.c.v.) (Fig. 2B), but not by a δ-opioid