Antidepressant Effect of Intracerebroventricularly Administered Deltorphin Analogs in the Mouse Tail Suspension Test

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Several studies have proposed δ opioid receptors as influential targets for developing novel antidepressants. Deltorphin (DLT) and deltorphin II (DLT-II) have high affinity and selectivity for δ opioid receptors; thus, it is likely that DLT analogs possess antidepressant-like effects. Based on this, we evaluated the effects of four DLT analogs (DLT-related synthetic peptides) on immobility behavior in the tail suspension test. Intracerebroventricular administration of DLT or [N-isobutyl-Glyδ]DLT in mice significantly decreased immobile behavior. However, administration of DLT did not affect locomotor activity, whereas that of [N-isobutyl-Glyδ]DLT significantly increased locomotion in mice. The effect of the shortened immobility time following DLT administration was counteracted by the administration of the selective δ1 opioid receptor antagonist 7-benzylidenenaltrexone, but not by the selective δ2 opioid receptor antagonist naltrexone. These findings suggest that DLT has an antidepressant-like effect by activating the central δ1 opioid receptor in mice.

Key words antidepressant; deltorphin; δ1 receptor

INTRODUCTION

The δ-opioidergic system in the brain may be directly involved in depression. Several studies have proposed δ opioid receptors as influential targets for developing novel antidepressants. In addition, pharmacological studies have shown that two δ opioid receptor subtypes are expressed in the nervous system and encoded by a single gene: the putative δ1 and δ2 opioid receptor subtypes. The δ receptor agonists SNC80 and KNT-127 and the selective δ2 opioid receptor agonist deltorphin II (DLT-II) have antidepressant-like effects in rodents. Moreover, another study reported that activation of the δ1 opioid receptor was involved in this antidepressant effect in mice. Hence, δ opioid receptor agonists may have the potential for use as novel antidepressant agents.

DLT, an endogenous heptapeptide isolated from the skin of the frog species Phyllomedusa bicolor and P. sauvagii, has high affinity and selectivity for δ opioid receptors. The δ opioid receptor affinity for [N-isobutyl-Glyδ]DLT, a δ-agonist/μ-antagonist, and [Ileδ5.6]DLT-II, a highly selective δ agonist, has been described by structure–activity relationship studies on DLTs. However, it remains unknown whether these analogs have antidepressant-like effects in vivo.

In this study, we examined the effects of DLT analogs on immobility behavior in the tail suspension test, thus evaluating the antidepressant-like effects of the molecules. In addition, we prepared scrambled-DLT and -DLT-II for use as negative controls in behavioral tests.

MATERIALS AND METHODS

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals from Tohoku Medical and Pharmaceutical University (Approval No. 18047-cn) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. We used the same animals used for the vehicle treatment and DLT treatment experiments for the tail suspension test to minimize suffering and reduce the number of animals used.

Animals Male ddY mice (weighing 28–32 g; Japan SLC, Shizuoka, Japan) were used for all experiments (n = 438). The mice were housed in cages with free access to food and water, under conditions of controlled temperature (22 ± 2 °C) and humidity (55 ± 5%) on a 12-h light–dark cycle (lights on: 07:00 to 19:00).

Drugs One of our colleagues synthesized the DLT analogs, viz. [N-isobutyl-Glyδ]DLT, [Ileδ5.6]DLT-II, scrambled-DLT, and scrambled-DLT-II. The amino acid sequences of the analogs are shown in Table 1. The DLT analogs were dissolved in Ringer’s solution containing 5% dimethyl sulfoxide (DMSO; Wako Pure Chemical Corporation, Osaka, Japan), while 7-benzylidenenaltrexone (BNTX; Research Biochemicals International, Natick, MA, U.S.A.) and naltrexone (NTB; Re-

Table 1. Amino Acid Sequence of DLT Analogs

| Peptides | Amino acid sequence |
|----------|---------------------|
| DLT | Tyr-β-Met-Phe-His-Leu-Met-Asp-NH₂ |
| DLT-II | Tyr-β-Ala-Phe-Glu-Val-Gly-NH₂ |
| [N-Isobutyl-Glyδ]DLT | Tyr-β-Met-Phe-His-Leu-isobuG-Asp-NH₂ |
| Scrambled-DLT | Asp-Phe-Leu-β-Met-Tyr-Met-His-NH₂ |
| [Ileδ5.6]DLT-II | Tyr-β-Ala-Phe-Glu-Ile-Ile-Gly-NH₂ |
| Scrambled-DLT-II | Gly-Phe-Val-β-Ala-Tyr-Glu-NH₂ |

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search Biochemicals International) were dissolved in Ringer’s solution containing 0.25% DMSO. The doses of BNTX and NTB used were adopted from previous reports. 15, 16 BNTX and NTB have been reported as selective δ₁ and δ₂ opioid receptor antagonists, respectively. 17, 18 All compounds were administered at a volume of 5 μL intracerebroventricularly (i.c.v.) using a 50 μL Hamilton microsyringe attached to a disposable 27-G needle under inhalatory diethyl ether anesthesia.

**Tail Suspension Test** The tail suspension test was performed as previously described to evaluate the antidepressant-like effect of the investigated DLT analogs. 19 Mice were taped from the tail tip and suspended 30 cm from the floor. An investigator who was blinded to the treatment assignments observed the immobility behavior of the animals for 10 min.

**Locomotor Activity** Locomotor activity was determined using the SUPERMEX multichannel activity-counting system (Muromachi Kikai Co., Ltd., Tokyo, Japan). The detailed description of the apparatus has been previously reported. 20 Locomotor activity was measured during the light phase, between 11:00 and 15:00. For adaptation, each mouse was placed in an activity box for 15 min prior to being injected with either vehicle, DLT, or [N-isobutyl-Gly₆]DLT, or co-administered with DLT and BNTX, and locomotor activity was recorded for 90 min.

**Statistical Analysis** The results of the experiments are expressed as mean ± standard error of the mean (S.E.M.). The significance of differences was determined by one- or two-way ANOVA, followed by the Tukey–Kramer test for multiple group comparisons. The criterion of significance was set at p < 0.05.

**RESULTS**

**Effects of DLT Analogs on Immobile Behavior during the Tail Suspension Test** To determine whether DLT analogs had antidepressant-like effects in vivo, we performed a tail suspension test and measured the changes in immobility time after administration of the analogs. Administration of DLT or [N-isobutyl-Gly₆]DLT to mice 30 min before the behavioral test significantly decreased the duration of immobility behavior compared to that in the vehicle group (DLT 0.1 nmol/mouse): p = 0.0312, Fig. 1(A); [N-isobutyl-Gly₆]DLT (1.0 nmol/mouse): p = 0.0110, Fig. 1(C)), while DLT-II, [Ile³⁶]DLT-II, and scrambled-DLT and -DLT-II did not show a statistical difference in immobility time when compared to the control group, suggesting that DLT and [N-isobutyl-Gly₆]DLT have potential antidepressant-like effects.

**Effects of DLT and [N-Isobutyl-Gly₆]DLT on Locomotor Activity in Mice** Mice were injected with vehicle, DLT, or [N-isobutyl-Gly₆]DLT, and locomotor activity was recorded for 90 min. The bars represent the mean ± S.E.M. One-way ANOVA: [F (2, 44) = 24.95, p < 0.0001]. Numbers in parentheses indicate the number of animals in each group. **p < 0.01, compared to the vehicle group (n = 10–25 per group).

**DISCUSSION**

In this study, we showed that DLT exhibited an antidepressant-like effect in mice through activation of the δ₁ opioid receptor.

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**Fig. 1.** Effects of DLT Analogs on Immobility Time in Mice

Vehicle or DLT analogs (i.c.v.) were administered 30 min before the tail suspension test. The bars represent the mean ± S.E.M. One-way ANOVA: [F (2, 45) = 4.006, p = 0.0251, Fig. 1(A); F (4, 72) = 3.852, p = 0.0068, Fig. 1(B); F (2, 61) = 5.282, p = 0.0077, Fig. 1(C); F (4, 66) = 0.272, p = 0.8950, Fig. 1(D); F (3, 61) = 2.274, p = 0.0889, Fig. 1(E); F (5, 60) = 1.059, p = 0.3735, Fig. 1(F)]. Numbers in parentheses indicate the number of animals in each group. *p < 0.05, compared to the vehicle group (n = 10–23 per group).

**Fig. 2.** Effects of DLT Analogs on Locomotor Activity in Mice

Mice were injected with vehicle, DLT, or [N-isobutyl-Gly₆]DLT, and locomotor activity was recorded for 90 min. The bars represent the mean ± S.E.M. One-way ANOVA: [F (2, 44) = 24.95, p < 0.0001]. Numbers in parentheses indicate the number of animals in each group. **p < 0.01, compared to the vehicle group (n = 10–25 per group).
Several studies have reported the antidepressant-like effects of δ opioid receptor agonists in mice. DLT is a heptapeptide and selectively binds to δ opioid receptors. Herein, we synthesized DLT analogs ([N-isobutyl-Gly\]^8\)DLT, [Ile^10\)DLT-II, scrambled-DLT, and scrambled-DLT-II) and examined whether these peptides exhibited antidepressant-like effects in vivo. We found that treatment with DLT or [N-isobutyl-Gly\]^8\)DLT significantly decreased immobility time in mice (Figs. 1(A), (C)). Moreover, DLT did not alter locomotor activity compared to the vehicle (Fig. 2). Based on these results, we can infer that DLT exerts an antidepressant-like effect in mice.

The two δ opioid receptor subtypes, δ₁ and δ₂, are highly expressed in the cortex, hippocampus, and striatum in response to mood regulation. 1,2) The δ receptor agonists SNC80 and KNT-127 have been reported to have antidepressant-like effects in naïve rodents 3,4) or olfactory bulbectomized rodents, which are an animal model of depression. 5,6) Moreover, the δ₂ receptor agonist DLT-II was observed to produce antidepressant-like effects in mice following a forced swimming test. 9) However, in the present study, we observed that DLT-II did not possess an antidepressant-like effect. These paradoxical results may be an artefact of the differences in the evaluation methods used in the two studies. To determine whether δ receptors are involved in the DLT-induced antidepressant-like effect, we investigated possible changes in the dopaminergic system in these regions of the brain producing an antidepressant-like effect in mice. 25,26)

Based on these reports, we hypothesize that the observed DLT-induced antidepressant-like effect may be associated with activation of the dopaminergic system in the brain that stimulates the δ₁ opioid receptor. Nevertheless, additional experiments to examine this hypothesis will be conducted and presented as future findings.

In conclusion, we demonstrated that injection of DLT directly into the cerebral ventricle decreased immobility time in mice via activation of the δ₁ opioid receptor, suggesting the antidepressant therapeutic potential of this δ₁ agonist. Therefore, DLT represents a new lead peptide for further investigation into the development of novel therapeutic approaches for depression.
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