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Note

Rubiscolin-6, a δ-Opioid Peptide from Spinach RuBisCO, Exerts Antidepressant-Like Effect in Restraint-Stressed Mice

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Summary Rubiscolin-6 (Tyr-Pro-Leu-Asp-Leu-Phe) is produced by a pepsin digest of spinach D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and known to act as an agonist on δ-opioid receptor. Here, we showed that administration of rubiscolin-6 reduced immobility time in the tail suspension test in restraint-stressed mice without effect on locomotor activity. The antidepressant-like effect of rubiscolin-6 was blocked by a δ-opioid receptor antagonist, naltrindole. These results indicate that rubiscolin-6 exerts antidepressant-like effect through activation of δ-opioid receptor.

Key Words rubiscolin-6, tail suspension test, depression, δ-opioid receptor, acute restraint stress

RuBisCO (D-ribulose-1,5-bisphosphate carboxylase/oxygenase) is found ubiquitously in photosynthetic organisms as an enzyme catalyzing carbon fixation reaction. RuBisCO is the most abundant protein on the earth since it occupies 10–30% of total leaf proteins (*1*, *2*). Rubiscolin-6 (Tyr-Pro-Leu-Asp-Leu-Phe) was originally isolated from a pepsin digest of spinach RuBisCO by using an opioid assay system in isolated mouse vas deferens (*3*). Rubiscolin-6 has been reported to have several actions in central nervous system (*4*). The peptide stimulated memory consolidation in passive avoidance experiment using step-through cages in mice (*5*). This effect was blocked by naltrindole, an antagonist of the δ-opioid receptor. Rubiscolin-6 also showed anxiolytic-like effect in mice using the elevated plus maze test (*6*). The effect was also blocked by naltrindole suggesting that these central nervous actions of rubiscolin-6 are mediated by δ-opioid receptor. Furthermore, the anxiolytic-like effect of rubiscoin-6 was blocked by SCH23390 and BMY14802, antagonists of the dopamine D_1 receptor and σ_1 receptor, respectively. These results indicate that the anxiolytic-like effect of rubiscolin-6 is mediated by both dopamine D_1 and σ_1 receptors downstream of δ-opioid receptor (*6*).

Delta-opioid receptor agonists have been proposed to be attractive targets for the development of novel antidepressants (*7*, *8*). However, there are no clinically used drugs for depression. Here, we evaluated the effects of rubiscolin-6 in the tail suspension test (TST), which has been widely used to investigate the antidepressant-like effect in mice (*9*). In this study, we also have used stressed mice exposed to an acute restraint stress, which was previously reported to induce an increase in detection sensitivity of antidepressant-like activity in the forced swimming test (FST) in mice (*10*).

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Materials and Methods

Male ICR mice (4 wk old, Charles River Japan, Atsugi, Japan) were housed at an ambient temperature of 23±2°C under a 12 h light/12 h dark cycle (lights on at 08:00) with free access to food and water. All animal procedures were performed in accordance with the institutional guidelines and approved by the Animal Care Committee at Hokuriku University (No. 17-03). Acute restraint stress was applied in tapered plastic film tubes (Mouse DecapiCones MDC-200; Braintree Scientific Inc., MA, USA) that allowed little movement. After introducing a mouse into a tube, the tube was fixed in horizontal position with vinyl tape, and left for 2 h. Immediately after restraint, they were moved back to the home cages. Non-stressed mice were placed separately in different cages for 2 h without food and water and then moved back to their home cages.

Rubiscolin-6 (YPLDLF) was synthesized using a continuous flow solid phase method with Fmoc-strategy by an automated peptide synthesizer (Model Pioneer; Thermo Fisher Scientific, MA, USA). The crude peptide was purified by a reverse-phase HPLC (Delta 600 HPLC system; Waters, MA, USA) on a column of Develosil ODS-HG-5 (2×25 cm; Nomura Chemical Co., Ltd., Seto, Japan). Naltrindole hydrochloride was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Each mouse was intraperitoneally (i.p.) administered with rubiscolin-6 or naltrindole dissolved in sterilized saline (10 mL/kg body weight) 1 h before the behavioral test.

The TST was originally described by Steru et al. (*11*), and was performed as described previously with a minor modification (*12*, *13*). The apparatus including testing

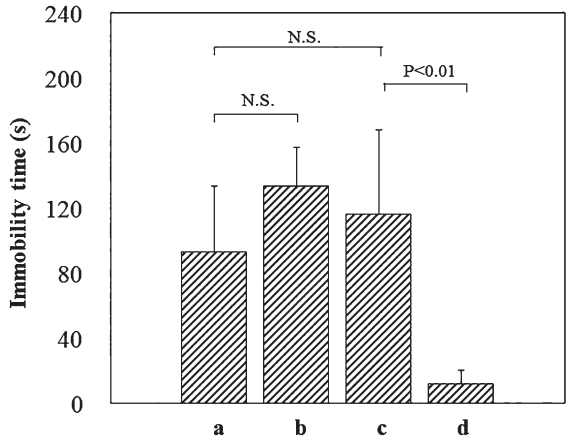

Fig. 1. Effects of rubiscolin-6 on immobility time in the tail suspension test in non-stressed and restraint-stressed mice. Rubiscolin-6 (30 mg/kg, i.p.) was administrated 1 h before the tests. Different lowercase letters indicate each experimental group: a, non-stress+saline; b, non-stress+rubiscolin-6; c, stress+saline; d, stress+rubiscolin-6. Values are represented as the means±SD. N.S., not significant.

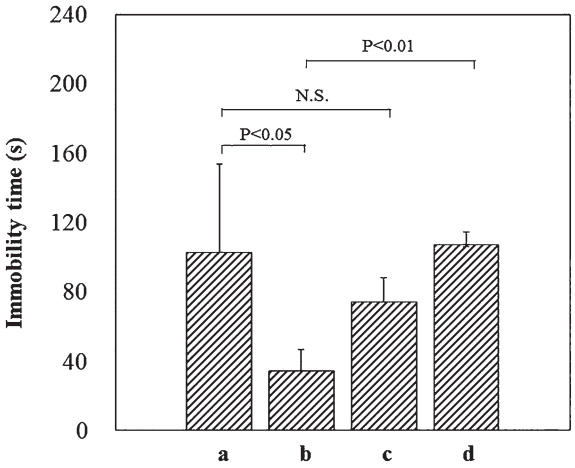

Fig. 2. Effects of naltrindole on the reduction of immobility time-induced by administration of rubiscolin-6 in restraint-stressed mice. Rubiscolin-6 (30 mg/kg, i.p.) was coadministered with naltrindole (1 mg/kg, i.p.) 1 h before the tests. Different lowercase letters indicate each experimental group: a, saline+saline; b, saline+rubiscolin-6; c, naltrindole+saline; d, naltrindole+rubiscolin-6. Values are presented as the means±SD. N.S., not significant.

Table 1. Effects of rubiscolin-6 on locomotor activity (LA) in non-stressed and restraint-stressed mice.

Restraint stress	Rubiscolin-6 (mg/kg)	LA counts (means±SD)
None	saline	5,418±1,763
None	30	5,055±1,397
Stress	saline	4,664±2,124
Stress	30	6,794±1,954

box (30 cm×25 cm×25 cm) and hooks used for suspending mouse were obtained from Yamashita Giken Co. (Tokushima, Japan). The mouse was suspended from a hook by an attachment, which was applied 25–30 mm from the tip of the tail. Each mouse was suspended for a total 6 min, and the duration of immobility was recorded during the last 4 min of the test. When mice climbed their tails up to the attachment or dropped from the attachment during the test session, the data were omitted. In the measurement of locomotor activity (LA), mice were individually placed in plastic chamber (40×40×40 cm) equipped with an automated activity monitoring system SCANET MV-20 (Melquest Ltd., Toyama, Japan). The LA was measured for 15 min. The behavioral tests were performed 1.5 h after acute restraint stress.

All results show one representative experiment out of two independent experiments and are given as means±SD (n=4–5). Data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey test. The analyses were performed with the statistical analysis system StatMate III (ATMS Co., Ltd., Tokyo, Japan). A probability value of less than 5% was considered statistically significant.

Results and Discussion

In the present study, acute restraint stress did not affect the immobility time in the TST (Fig. 1). Rubiscolin-6 administered at the dose of 30 mg/kg, which was ineffective in non-stressed mice, significantly reduced immobility time in restraint-stressed mice (Fig. 1). Rubscolin-6 did not significantly alter locomotor activity in both non-stressed and restraint-stressed mice (Table 1). Therefore, rubiscolin-6 exerts antidepressant-like effect in restraint-stressed mice. The dose of rubiscolin-6 used in this study was the maximal effective dose for antidepressant-like activity based on reduction of immobility time in the TST in the stressed mice (data not shown). We employed naltrindole, a δ-opioid receptor antagonist, to confirm whether the opioid receptor participates in the antidepressant-like effect of rubiscolin-6. The antidepressant-like effect by administration of rubiscolin-6 in restraint-stressed mice was significantly blocked by naltrindole as shown in Fig. 2. Naltrindole alone did not alter immobility time in the TST.

Various kinds of bioactive peptides have been isolated from enzymatic digests of natural proteins of animal and plant origin. Rubiscolins are two naturally occurring, Tyr-Pro-Leu-Asp-Leu (rubiscolin-5) and rubiscolin-6, isolated from the pepsin digests of RuBisCO from spinach leaves (*3*). Rubiscolin-6 was shown to possess memory enhancing and anxiolytic-like activities in mice (*5*, *6*). These action of the peptide probably appeared through the agonistic action on δ-opioid receptor. Our study here indicates that rubiscolin-6 has also antidepressant-like effect, which is mediated by δ-opioid receptor.

Depression is a main cause of distress worldwide. More than 300 million people across the world suffer from depression which accounts for 4.4% of the world's

population. There has also been an 18% increase in cases of depression from 2005 to 2015, worldwide (*14*). Current prevention and treatment strategies for depression include non-pharmacological and pharmacological treatment. The selective serotonin reuptake inhibitors (SSRIs) and the serotonin and norepinephrine reuptake inhibitors (SNRIs) were the first choice for pharmacological treatment of clinical depression. Most of antidepressants have been developed based on the monoamine hypothesis of depression (*15*). These antidepressants are thought to immediately increase synaptic concentrations of monoamines in brain. However, it is recognized that it takes time to exert their therapeutic effects as antidepressants. Recently, δ-opioid receptor agonists have been proposed to be attractive targets for the development of novel antidepressants (*7*, *8*). There are, however, the preclinical studies that a portion of non-peptidic δ-opioid receptor agonists produced convulsive effects (*16*). On the other hand, bioactive peptides such as rubiscolin-6 are thought to be relatively safe due to food origin. Therefore, our results may give a precious idea on strategy for discovery of a novel type of antidepressant and on complementary therapies in the treatment of depression.

In the present study, we show that rubiscolin-6 exerts antidepressant-like effect in restraint-stressed mice, and that this effect is blocked by naltrindole. It has been reported that acute restraint stress-induced increase in immobility time in the FST is more sensitive for detection of antidepressant-like activity of imipramine, which is known to inhibit both serotonin and norepinephrine reuptake (*10*). Several studies showed that the tissue concentration of serotonin and its metabolite 5-hydroxyindole acetic acid in olfactory bulbectomized rats, an animal model of depression, are decreased in the mid-brain and limbic-cortical areas, such as frontal cortex, hippocampus and amygdala (*17*). These decreases were normalized by SNC80, a non-peptide selective δ-opioid receptor agonist (*18*). Taken together, our results suggest that the antidepressant-like activity of rubiscolin-6 could be mediated by modulation of monoaminergic neurotransmission downstream of δ-opioid receptor in central nervous system.

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