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Neoechinulin A induced memory improvements and antidepressant-like effects in mice☆

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ABSTRACT

Neoechinulin A is an isoprenyl indole alkaloid that exhibits scavenging, neurotrophic factor-like, and anti-apoptotic activities. However, the effectiveness of neoechinulin A in animal models of disease has not yet been explored. In the present study, we investigated the effects of neoechinulin A on memory impairment in lipopolysaccharide (LPS)-treated mice and its antidepressant-like effects in mice. In the Y-maze test, the intracerebroventicular (i.c.v.) administration of LPS (10 μg/mouse) significantly decreased spontaneous alternation behavior, which was prevented by the prior administration of neoechinulin A (300 ng/mouse, i.c.v.). None of the treatments altered the locomotor activity of mice. Moreover, the administration of neoechinulin A decreased the immobility time in the forced-swim test or tail suspension test, which was prevented by the prior administration of WAY100635 (an antagonist of 5-HT_{1A} receptors) and parachlorophenylalanine (an inhibitor of tryptophan hydroxylase). These results suggest that neoechinulin A improves memory functions in LPS-treated mice, and also exerts antidepressant-like effects through changes in the 5-HT system.

1. Introduction

Neoechinulin A is an alkaloid consisting of three structural moieties: an indole, diketopiperazine, and isoprenyl moiety (Fig. 1). It was first isolated from *Aspergillus amstelodami* (Dossena et al., 1974) and described as a secondary metabolite from *Eurotium* species (Slack et al., 2009). Neoechinulin A was previously shown to exhibit anti-proliferative activity in various tumor cells (Pettit et al., 2008), exert protective effects in neuronal cells (Akashi et al., 2011; Kimoto et al., 2007; Maruyama et al., 2004), exhibit anti-oxidant activity (Yagi and Doi, 1999), radical-scavenging activity (Li et al., 2004), ultraviolet-A-protecting activity (Li et al., 2004), and pro-inflammatory activity in the lungs (Miller et al., 2010), and anti-neuroinflammatory activity in murine microglia BV2 cells (Dewapriya et al., 2013).

Neuroinflammatory responses are reportedly induced in a number of neurodegenerative disorders including Alzheimer's disease (AD) (Barone and Feuerstein, 1999; McGeer et al., 1989), in the early stage of which the production of several cytokines and chemokines in glia play important roles (Kielian, 2004). Lipopolysacchride (LPS), an endotoxin found in the outer leaflet of the outer membrane of Gram-negative bacteria, activates macrophages to produce pro-inflammatory mediators and pro-inflammatory cytokines, and also affects learning and memory (Cunningham and Sanderson, 2008). Although a previous study demonstrated the anti-inflammatory effects of neoechinulin A in LPS-stimulated RAW264.7 macrophages (Kim et al., 2013), its effects on cognitive behavior remain unknown.

Neuropsychiatric symptoms, such as anxiety and depression, are frequently reported in patients with cognitive decline and dementia (Alexopoulos et al., 1997; Lyketsos and Olin, 2002; Tiemeier, 2003). For example, between 20% and 50% of AD patients have depression (Fernandez-Martinez et al., 2010; Olin et al., 2002; Vilalta-Franch et al., 2006). Previous studies on the pathophysiology of depression in AD have focused on serotonergic transmission. Reduced 5-HT_{1A} receptor expression was reported to be specifically correlated with depressive symptoms in AD (Lai et al., 2011). In addition, a relationship has

Abbreviations: 5-HT, 5-hydroxytryptamine; AD, Alzheimer's disease; ANOVA, analysis of variance; DMSO, dimethylsulfoxide; FST, forced-swim test; i.c.v., intracerebroventicular; i.p., intraperitoneal; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; s.c., subcutaneous; TST, tail suspension test; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide; NF-κB, transcription factor-κB; PBS, phosphate-buffered saline; PCPA, 4-chloro-DL-phenylalanine; SSRI, selective serotonin reuptake inhibitors.

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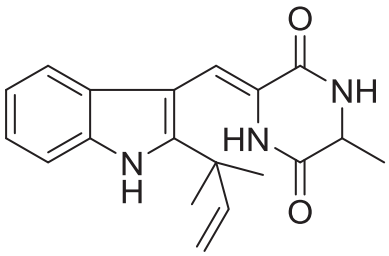

Fig. 1. Chemical structure of neoechinulin A.

been also identified between major depression in AD and 5-HT_{2A} and 5-HT_{2C} receptor polymorphisms (Holmes et al., 2003).

In the present study, we determined whether neoechinulin A induces memory improvements and exerts antidepressant-like effects. The brain serotonin (5-hydroxytryptamine; 5-HT) system has been strongly implicated in the neural regulation of mood and anxiety states. We thus also evaluated the involvement of the 5-HT system in the antidepressant-like effects of neoechinulin A.

2. Materials and methods

2.1. Preparation of neoechinulin A

Neoechinulin A was prepared as described previously (Kuramochi et al., 2008).

2.2. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science, and were conducted according to the guidelines of the National Institute of Health and Japan Neuroscience Society. We used six-week-old male ddY mice (Japan SLC, Shizuoka, Japan), and attempted to minimize the number of animals used and their suffering. All animals were kept in a controlled environment, with a 12:12-h light schedule, temperature (23 °C), and relative humidity (55 ± 5%) for at least 5 days before the experiments, and were provided ad libitum access to food and water.

2.3. Neoechinulin A administration

Neoechinulin A was dissolved in 0.1% dimethylsulfoxide (DMSO) (Wako Pure Chemical Industries, Osaka, Japan). Neoechinulin A (30 ng or 300 ng) or 0.1% DMSO (vehicle control) was administered into the lateral ventricular region of the mouse brain because the ability of neoechinulin A to penetrate the blood-brain barrier remains unknown. The intracerebroventicular (i.c.v.) administration (a volume of 5 μl/mouse) was performed under brief ether or isoflurane anesthesia according to the method of Haley and McCormick (1957). Since previous in vitro studies have shown that an incubation with neoechinulin A (50–200 μM) for 24 h protected cultured neurons from cell death induced by neurotoxic mediators (Akashi et al., 2011; Kimoto et al., 2007; Maruyama et al., 2004), neoechinulin A was administrated 24 h before behavioral experiments were performed in this study. Furthermore, the dose of neoechinulin A (30 or 300 ng, i.c.v.) administered was determined based on these previous studies.

2.4. Spontaneous alternation behavior

2.4.1. Y-maze test

The experimental schedule is shown in Fig. 2A1. The Y-maze test was performed under bright (fluorescent room light) conditions, and as described previously with some modifications (Sasaki-Hamada et al., 2014). 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 were recorded visually. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The effect was calculated as the percent alternation according to the following formula: Percent alternation = {(number of alternations)/(total number of arm entries − 2)} × 100 (%). The arms were wiped down with paper between sessions.

2.4.2. Spontaneous alternation behavior in LPS-treated mice

The experimental schedule is shown in Fig. 2B1. LPS (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.01 M phosphate-buffered saline (PBS). Y-maze test sessions were carried out 3 days after the LPS treatment (10 μg, i.c.v.), which was described previously (Iwai et al., 2014, 2015).

2.5. Open-field test

The experimental schedule is shown in Fig. 3A. The open-field test was performed using a modification to the procedure described in our previous study (Iwai et al., 2015). The open-field apparatus consisted of a square area (40 × 40 cm) with 25-cm-high opaque walls. The floor was divided into 16 equal squares by lines. The central area comprised the central 4 squares (20 × 20 cm). Mice were placed in a corner of the open-field facing the opaque walls. The number of crossings in the central area and the total number of crossings were then counted for 5 min. The apparatus was wiped down with paper after the removal of each animal.

2.6. Forced-swim test and drug treatment

The forced-swim test (FST) was performed as described previously (Iwai et al., 2013; Sasaki-Hamada et al., 2015). The test was performed by placing a mouse in an acrylic cylinder (50 cm in height, 18 cm in diameter) containing a 7-cm water column (25 ± 1 °C). The water was replaced between every trial. Two swimming sessions were conducted on Day −1 and −2 (Fig. 4A): an initial 15-min pretest, followed by a 6-min test 24 h later. On the day of the FST session, neoechinulin A or vehicle was administrated 30 min before the pretest. Test sessions were recorded through a web-camera system on a hard disk in order to measure the time of immobility, with immobility being defined as passive floating passively in the water and only making slight movements to keep the head above the water line. The scored immobility time was blindly checked by the co-authors. The following drugs were used: ketanserin tartrate salt, 4-chloro-DL-phenylalanine (PCPA), and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl) cyclohexanecarboxamide (WAY100635) (Sigma-Aldrich). All drugs were dissolved in PBS. PCPA (150 mg/kg) was intraperitoneally (i.p.) pretreated once a day for 4 consecutive days. Twenty-four hours after the last PCPA treatment, mice were administered neoechinulin A or vehicle, and the FST was then performed as described above. WAY100635 (0.1 mg/kg) was administered subcutaneously (s.c.) 30 min, and ketanserin (5 mg/kg) was administered i.p. 45 min before the second swimming session, respectively. The dosage, time schedules and routes of drug administration were based on previous studies (Mayorga et al., 2001; Iwai et al., 2009).

2.7. Tail suspension test

Neoechinulin A or vehicle was administrated 24 h before the tail suspension test (TST). Mice were individually suspended by the tail from a horizontal ring (distance from the floor = 27 cm) in a gray acrylic box (30 × 15 × 15 cm) (Bio Research Center, Nagoya, Japan) using adhesive tape affixed 2 cm from the tip of the tail. A 5-min test session was employed under bright (fluorescent room light) conditions, and was recorded through a web-camera system on a hard disk. The behavioral parameter measured was the time of immobility, which was defined as the