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Subchronic administration of rosmarinic acid, a natural prolyl oligopeptidase inhibitor, enhances cognitive performances

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ABSTRACT

It is well known that inhibition of prolyl oligopeptidase (POP) is involved in memory-related function. In this study, we observed that rosmarinic acid (RA) inhibits POP activity with an IC_{50} of 63.7 μM. Subsequently, we investigated the cognitive-enhancing effects of RA employing the Morris water maze paradigm. The results demonstrated that RA is non-competitive POP inhibitor and that acute and subchronic RA treatments showed an inverted U-shaped dose–response curve in the platform crossings. Furthermore, chronic RA treatment significantly increased the platform crossings. These results suggest that RA has a cognitive-enhancing effect which may be mediated by inhibition of POP.

1. Introduction

Prolyl oligopeptidase (POP, E.C. 3.4.21.26), an 80 kDa serine protease that cleaves several neuropeptides comprised 30 or less amino acid residues at the carboxyl side of their internal proline residue. These include arginine-vasopressin (AVP), substance P (SP), oxytocin, and angiotensin IV [1,2]. In addition to their physiological functions, several studies have indicated that these peptides play roles in neurological functions [3–7]. For example, clinical and preclinical studies have demonstrated that AVP is involved in memory consolidation, storage and retrieval, as well as social behaviors and fear responses [3–6]. In addition, SP, a neurokinin, is also known to modulate cognitive function. Systemically or intracerebrally injected SP prevents aging-related memory deficits and enhances cognitive performance in animal models, which is mediated by the increase of acetylcholine in the frontal cortex [7]. Central infusion of angiotensin IV is also involved in cognitive processes [1]. Thus, an elevation in levels of AVP, SP, and angiotensin IV in the brain by the inhibition of POP activity may enhance cognitive function. We screened several hundred natural compounds and found that rosmarinic acid (RA) has an inhibitory effect on POP activity. RA is a polyphenolic compound found in various species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. It was previously characterized for its anti-oxidative, anti-inflammatory, anti-mutagenic, anti-bacterial and anti-viral effects [8].

We hypothesized that RA, as a POP inhibitor, might enhance cognitive function or improve the symptoms of psychatric disorders such as affective disorders [9]. Here, we demonstrate that RA may enhance cognitive performance in vivo using the Morris water maze task.

2. Materials and methods

2.1. Animals

Male ICR mice (25–30 g) were purchased from the Orient Co., Ltd., a branch of Charles River Laboratories (Seoul, Korea). Animals were housed 5 per cage with food and water available ad libitum and maintained under a constant temperature

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(23 ± 1 °C) and humidity (60 ± 10%) under a 12-h light/dark cycle (light on 07:30–19:30 h). All behavioral tasks were conducted between 10:00 h and 16:00 h. Animal treatment and maintenance were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) and the Animal Care and the Use Guidelines of Kyung Hee University, Korea.

2.2. Materials

Rosmarinic acid, berberine, bacitracin and Z-glycyl-L-proline-4-nitroanilide were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Bicinchoninic acid (BCA) protein assay reagent was purchased from Thermo Fisher Scientific (Rockford, IL). All other materials were of the highest grade available and were obtained from normal commercial sources.

2.3. Drug administration

RA was dissolved in 0.9% saline. Mice were given oral doses of RA (1, 2, 4, or 8 mg/kg) for acute (4 training days) or subchronic periods (2 or 3 weeks). The oral administration period included experimental periods for training trials.

2.4. Prolyl oligopeptidase inhibition assay

POP activity was determined using Z-glycyl-L-proline-4-nitroanilide as a substrate as previously described [10,11]. Whole brains were homogenized in 3 volumes of 0.1 M sodium–potassium phosphate buffer (pH 7.0) and centrifuged at 14,000 × *g* for 20 min, and the supernatant was used as the enzyme source. Z-glycyl-L-proline-4-nitroanilide was dissolved in 1,4-dioxane to yield a 40 mM solution. Enzyme solution (10 μl), sodium–potassium phosphate buffer (0.1 M, pH 7.0, 457.5 μl), and various concentrations of test compounds in sodium–potassium phosphate buffer solution (10 μl) were mixed and preincubated for 15 min at 30 °C. The reaction was initiated by adding Z-glycyl-L-proline-4-nitroanilide (40 mM, 2.5 μl), and the reaction mixture was incubated for 60 min at 30 °C. Distilled water (10 μl) was added instead of the test compound solution as a control. Additionally, 1,4-dioxane (2.5 μl) was added instead of the substrate solution as the blank. 4-Nitroanilide, the product of the reaction, was measured colorimetrically at a wavelength of 410 nm (Molecular devices, Sunnyvale, CA). The enzyme activity curve was obtained by plotting the percentage of enzyme activity (100% for the control) versus the logarithm of test compound concentrations. In order to determine the type of inhibition, various concentrations of Z-glycyl-L-proline-4-nitroanilide were used, and 4-nitroanilide formation was monitored every 4 min at 30 °C. The protein content of the samples was measured using BCA protein assay reagent [12].

2.5. Morris water maze task

Spatial memory was assessed by the Morris water maze task based on the method described by Morris [13]. The tasks were conducted in a circular metal pool (90 cm in diameter and 45 cm high), filled with water and maintained at 20–25 °C and made opaque by black food coloring. The pool was virtually divided into four equal quadrants, and one of them was allocated as the target quadrant. Four visual cues were placed at the perimeter of each quadrant. A white platform (diameter, 6 cm; height, 30 cm) was placed in the center of the target quadrant. The water level in the pool was adjusted so that the platform was submerged 1 cm below the surface of the water. The position of the platform was fixed throughout the training trials. The animals were trained for 60 s without the platform on the first day. In the next four days, they were given one training trial per session per day, rather than two or four trials per session to avoid rapid learning of the maze paradigm. During the training trial session, the mice were placed in the water, facing the pool wall in one of the pool quadrants and allowed to navigate the pool for 120 s in order to find the platform. If the animals found the platform and stayed on the platform for 5 s, then they were removed from the pool and allowed to dry under an infrared lamp. If they failed to find the platform, the mice were guided and placed onto the platform for 10 s. The starting position was changed randomly each day. The time required to find the platform, which is termed escape latency, was measured with a stopwatch. Mice were orally administered vehicle or RA (1, 2, 4, or 8 mg/kg) 60 min before each training trial. The final administration was done on the 4th training trial day. One day after the final training session, the mice were subjected to a probe trial session in which the platform was removed from the pool. Mice were allowed to swim for 120 s to search for it. Swimming behaviors were recorded by a video camera mounted above the center of the pool and connected to a computer. The recordings were analyzed using an automated tracking system Ethovision System (Nodulus, Wageningen, The Netherlands) for path length, time spent in the target quadrant, the number of platform crossings, and speed.

2.6. Statistical analysis

Data from the Morris water maze task was analyzed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for multiple comparisons. All statistical analyses were processed using Sigmastat software (Systat Software, IL 60606). The enzymatic assay data were analyzed using the GraphPad Prism (GraphPad Software, CA 92037) to yield IC_{50} value. Statistical significance was accepted for P values of <0.05.

3. Results

3.1. Rosmarinic acid inhibits prolyl oligopeptidase

We assessed the inhibitory effect of RA on POP in vitro. RA inhibited POP in a dose-dependent manner with an IC_{50} of 63.7 μM (Fig. 1A). Berberine and bacitracin, which were previously reported as POP inhibitors [11,14], were also evaluated as positive controls. Their IC_{50} values were 218.3 and 9.2 μM, respectively. The Lineweaver–Burke plot also revealed that the mode of inhibition of RA was non-competitive (Fig. 1B).

3.2. Subchronic rosmarinic acid treatment enhances cognitive performance in the Morris water maze task

The Morris water maze task was used to assess the effects of RA on spatial memory [13]. The mice were given an oral administration of vehicle (0.9% saline solution) or several doses of RA (1, 2, 4, or 8 mg/kg) for various periods (4 days, 2