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Effects of *Hericium erinaceus* on amyloid β(25-35) peptide-induced learning and memory deficits in mice

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ABSTRACT

The mushroom *Hericium erinaceus* has been used as a food and herbal medicine since ancient times in East Asia. It has been reported that *H. erinaceus* promotes nerve growth factor secretion *in vitro* and *in vivo*. Nerve growth factor is involved in maintaining and organizing cholinergic neurons in the central nervous system. These findings suggest that *H. erinaceus* may be appropriate for the prevention or treatment of dementia. In the present study, we examined the effects of *H. erinaceus* on amyloid β(25-35) peptide-induced learning and memory deficits in mice. Mice were administered 10 μg of amyloid β(25-35) peptide intracerebroventricularly on days 7 and 14, and fed a diet containing *H. erinaceus* over a 23-d experimental period. Memory and learning function was examined using behavioral pharmacological methods including the Y-maze test and the novel-object recognition test. The results revealed that *H. erinaceus* prevented impairments of spatial short-term and visual recognition memory induced by amyloid β(25-35) peptide. This finding indicates that *H. erinaceus* may be useful in the prevention of cognitive dysfunction.

The mushroom *Hericium erinaceus* (Lion's mane or Yamabushitake) has been used as a food and herbal medicine since ancient times in East Asia. Hericenones C-H (8, 9, 12) and erinacines A-I (10, 11, 15), compounds promoting nerve growth factor (NGF) synthesis in cultured astrocytes, were isolated from the fruiting body and mycelium of *H. erinaceus*, respectively. NGF primarily acts on cholinergic neurons in the central nervous system and has potent biological effects, such as the induction of neuronal differentiation, the promotion of neuronal survival, and regeneration (20). Studies using several animal models of cognitive dysfunction have reported that NGF ameliorates neurodegeneration and cognitive deficits (1, 3, 13, 22). On the basis of these findings, it has been proposed that NGF may be appropriate in the treatment of neurodegenerative diseases including Alzheimer's disease (24). Previous reports have proposed that *H. erinaceus* may be useful for the treatment and/or prevention of dementia. In accord with this notion, we demonstrated previously that *H. erinaceus* extract induced NGF mRNA expression in 1321N1 human astrocytoma cells (19), and that oral administration of *H. erinaceus* increased NGF mRNA expression in the mouse hippocampus (19). Furthermore, scores on the cognitive function scale were improved by *H. erinaceus* in patients suffering from mild cognitive impairment (18).

Alzheimer's disease is the most common cause of senile dementia. It is associated with notable degeneration and loss of cholinergic neurons in the basal forebrain, which is correlated with the formation of senile plaques and neurofibrillary tangles (2, 27). The main component of senile plaques is the amyloid β (Aβ) peptide which comprises 39-42 amino acids (5, 6, 23). Several reports have demonstrated

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that intracerebral injections of Aβ-related peptide impair the learning and memory in rodents (4, 16, 17). In addition, one study reported that the 11-amino acid fragment of Aβ, Aβ(25-35) also has potent amnesic effects, and that the Aβ(25-35) region in the Aβ peptide is involved in neurotoxicity accompanied by aggregation (21). Moreover, administration of Aβ(1-40), Aβ(1-42), and Aβ(25-35) peptides has been found to impair learning and memory to the same extent in rodents (25).

In the present study, we investigated the effects of oral administration of *H. erinaceus* on Aβ(25-35)-induced cognitive impairment in mice. Aβ(35-25), a reversed sequence of Aβ(25-35), was used as a control peptide. Learning and memory function of mice was evaluated with behavioral pharmacological methods including the Y-maze test and the novel-object recognition test.

MATERIALS AND METHODS

Materials. Aβ(25-35) was purchased from Wako Pure Chemicals (Tokyo, Japan). Aβ(35-25) was sourced from Bachem AG (Bubendorf, Switzerland). Fruiting bodies of *H. erinaceus* were cultivated by the Hokuto Corporation (Nagano, Japan).

Animals. Male 5-week-old ICR mice (Nihon SLC Inc, Japan) were used in the experiment. The animals were provided with free access to food and water, and were maintained under controlled conditions at a temperature of 24 ± 1°C, relative humidity of 45 ± 5% and a 12-h: 12-h light-dark cycle (light period, 9 : 00–21 : 00). The experimental procedures were performed in accordance with the guidelines of the Institution for Animal Care and Use Committee of Tohoku University.

Administration of Aβ(25-35) and experimental design. At the start of experiment (day 0), animals were divided into four groups: treatment with Aβ(35-25) and a control diet, treatment with Aβ(35-25) and a *H. erinaceus* diet, treatment with Aβ(25-35) and a control diet, treatment with Aβ(25-35) and a *H. erinaceus* diet. The average weight was equalized between groups. Fruiting bodies of *H. erinaceus* were lyophilized and powdered just after harvest. The powder of *H. erinaceus* was mixed with a normal powdered diet (MF; Oriental Yeast, Tokyo, Japan), adjusted to contain 5% (w/w) *H. erinaceus*. The control diet group was given a normal diet containing 5% dextrin for 23 d.

Aβ(25-35) peptide and Aβ(35-25) peptides were administered by intracerebroventricular injection following a method established by Laursen and Belknap (14). Aβ peptides were dissolved in sterile saline at a concentration of 1 mg/mL and stored at −30°C until use. The peptides were then incubated for 4 d at 37°C. Using a light microscope we observed that Aβ(25-35) peptide, but not Aβ(35-25) peptide, formed fibril structures before administration (data not shown). The peptide solution (10 μL) was administered intracerebroventricularly on day 7 and day 14 after the start of the test period using the following method: each mouse was lightly anaesthetized with diethyl ether, and a 50-μL Hamilton microsyringe equipped with a 28-gauge needle was inserted to a depth of 2.5 mm unilaterally 1 mm lateral to bregma. Peptide was injected gradually over a period of approximately 10 s. All mice exhibited normal behavior within 3 min after administration. The administration site was confirmed by injecting trypan blue into the lateral ventricle of the mice in preliminary experiments.

Y-maze test. Spatial short-term memory was assessed using the Y-maze test. The Y-maze test was carried out on day 21 after the start of test period. The maze floor and walls were constructed from black acrylic plastic. Each arm was 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top, and converged at an equilateral triangular central area. The testing procedure was based on a method described by Huang *et al.* (7). Each of the mice was positioned at the end of one arm and allowed to explore the Y-maze freely for a period of 8 min. The sequence and number of arm entries were recorded manually. An arm entry was considered to be complete when the hind paws of the mouse had all entered the whole way into an arm. An alternation was defined as the entry into all three arms on consecutive choices (ABC, ACB, BAC, BCA, CAB and CBA). The percentage of alternation was determined by dividing the total number of alternations by the total number of arm entries minus 2 multiplied by 100 as shown in the following equation: Alternation (%) = [(number of alternation) / (total arm entries − 2)] × 100. Mice which did not reach nine arm entries within 8 min were excluded from further analysis.

Novel-object recognition test. Visual recognition memory was examined using the novel-object recognition test. The novel-object recognition test was carried out on day 21–23 based on the procedure described by Tordera *et al.* (26). The apparatus con-

sisted of an open square box (30 × 40 × 30 cm) constructed from dark green plastic. The objects to be discriminated were made of inert material such as glass, plastic or metal. The novel-object recognition test was started on day 21. During the first two consecutive days (days 21 and 22), mice were individually put into the empty box for 10 min to habituate to the apparatus. On day 23, the object recognition task was carried out as follows: two identical objects (A1 and A2) were placed symmetrically in the box at a distance of 20 cm from each other. Each mouse was placed in the box and exposed to the objects for 10 min (sample phase), then returned to its cage. After a 1-h delay, the mouse was placed back in the box and exposed to the familiar object (A3) and a novel object (B1) for 10 min (choice phase). An experimenter, who was naive to the group identity of the individual mouse, recorded the total time spent exploring each of the two objects. Exploration was defined as directly attending to the object with the head at a distance less than 2 cm from the object. The discrimination ratio was calculated as the time spent exploring the novel object divided by total time spent exploring both the novel object and the familiar object: discrimination ratio (%) = [B1 / (A3 + B1)] × 100. A higher discrimination ratio reflected successful discrimination, and was regarded as demonstrating superior memory function enabling the mouse to recognize the familiar object.

Statistical analysis. Data were expressed as means ± SEM. Significant differences were determined by one-way ANOVA followed by a Tukey's test (Fig. 1 and Fig. 2B) or Student's paired *t*-test (Fig. 2A).

RESULTS AND DISCUSSION

To investigate the effect of *H. erinaceus* on memory and learning of mice with an Aβ(25-35)-induced cognitive impairment, we fed mice on a test diet containing 5% dried *H. erinaceus* for 23 d, and examined their behavior using the Y-maze test and the novel-object recognition test. There was no significant difference in food intake among the four groups. The food intake average through the experimental period was approximately 5.4 g/d/mouse in all groups.

Mice were tested in the Y-maze at day 21 of the experimental period. Spontaneous alternation behavior reflects spatial memory capacity, relying on the ability of animals to enter an arm of the Y-maze that was not entered in the previous choices. In the mice fed on a control diet, the administration of aggregated Aβ(25-35) into the cerebral ventricle resulted in a significant decrease in alternation behavior. However, in mice fed on an *H. erinaceus* diet, there was no significant difference in alternation behavior between the Aβ(25-35) group and the Aβ(35-25) group (Fig. 1A). *H. erinaceus* did not affect the alternation behavior of control mice administered with Aβ(35-25). The total number of entries did not differ significantly among the four different treatment groups of mice (Fig. 1B). Therefore, the difference in alternation behavior could not be attributed to exploratory, locomotor or motivational effects.

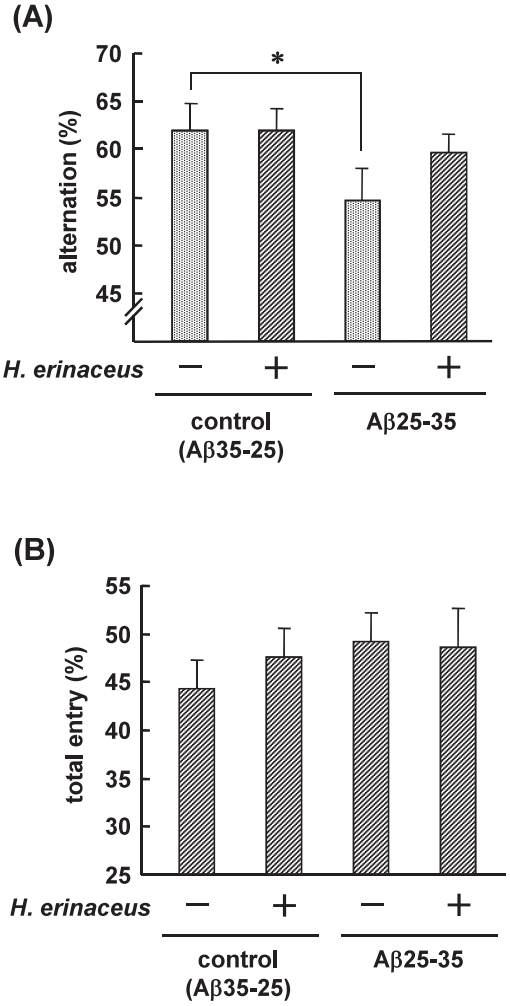

Fig. 1 Effects of *H. erinaceus* on A*β*(25-35)-induced memory deficits in the Y-maze test. (A) Spontaneous alternation behavior: Alternation (%) = [(number of alternations) / (total arm entries-2)] × 100. (B) The number of total arm entries during an 8 min-test period. Values represent the means ± SEM. n = 18 (A*β*(35-25) and control diet), 15 (A*β*(35-25) and *H. erinaceus* diet), 19 (A*β*(25-35) and control diet), 13 (A*β*(25-35) and *H. erinaceus* diet). *$P < 0.01$ vs. A*β*(35-25) and control diet group (Tukey's test).

A novel-object recognition test was carried out at days 21–23. During the sample phase, the four groups explored each objects (A1 and A2) to the same extent (data not shown). During the choice phase, in the group fed on a control diet, control mice (administered with Aβ(35-25)) spent more time exploring the novel object than the familiar object. In contrast, mice administered with Aβ(25-35) showed no significant difference in the time spent exploring the two objects (familiar and novel; Fig. 2A). Moreover, the discrimination ratio was significantly decreased by the administration of Aβ(25-35). On the other hand, in mice administered both Aβ(25-35) and *H. erinaceus*, exploration time was significantly longer for the novel object than the familiar object, and the discrimination ratio was significantly higher than that of the mice treated with Aβ(25-35) and fed on a control diet (Fig. 2B). The total time spent exploring did not differ significantly between the four different groups (data not shown). These results suggest that mice treated with Aβ(25-35) were impaired in visual recognition memory, and failed to discriminate the familiar object, and *H. erinaceus* prevented the Aβ(25-35)-induced impairment.

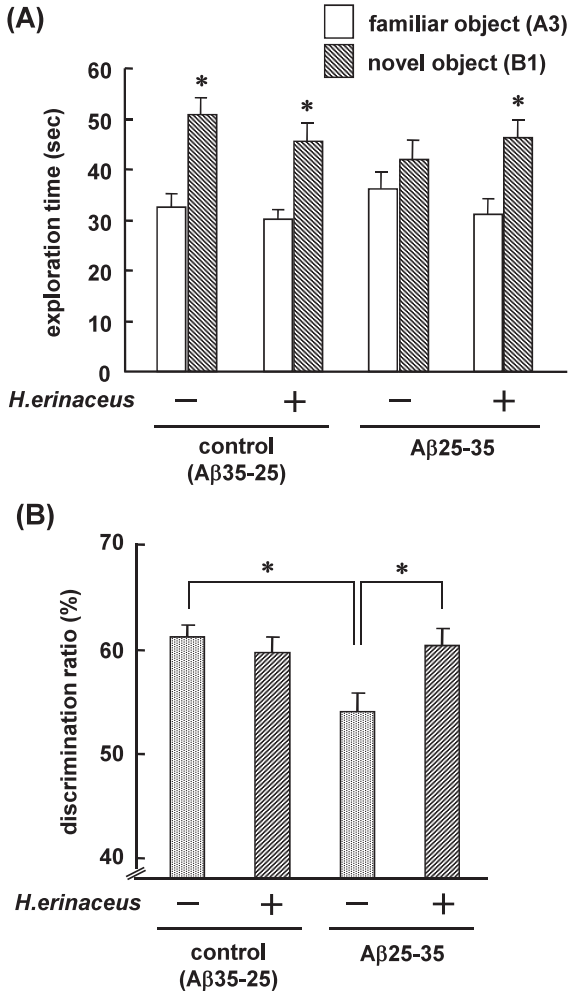

Fig. 2 Effects of *H. erinaceus* on A*β*(25-35)-induced memory deficits in the novel-object recognition test. Mice were exposed to two identical objects (A1 and A2) for 10 min (sample phase). After a 1 h delay, the mice were exposed to the familiar object (A3) and a novel object (B1) (choice phase). (A) Total time spent in exploring each object type (A3 and B1) during the test phase. (B) Discrimination ratio (%) = [B1 / (A3 + B1)] × 100. Values represent the means ± SEM. n = 20 (A*β*(35-25) and control diet), 18 (A*β*(35-25) and *H. erinaceus* diet), 21 (A*β*(25-35) and control diet), 20 (A*β*(25-35) and *H. erinaceus* diet). **P* < 0.01 vs. A*β*(35-25) and control diet group (Student's paired *t*-test (A) or Tukey's test (B)).

Regarding the active substances in *H. erinaceus*, it was reported that hericenones C-H isolated from fruiting body of *H. erinaceus* promoted NGF synthesis in astrocytes (9, 12). The current results confirmed the presence of hericenone C, D, and E (Fig. 3) in the *H. erinaceus* used in our study. Using HPLC, the freeze-dried fruiting bodies of *H. erinaceus* were found to contain 4.12 mg/g of hericenone C, 0.21 mg/g of hericenone D, and 0.49 mg/g of hericenone E (Mori *et al*., unpublished observations). Furthermore, we found evidence suggesting the possibility of other active compounds, which are

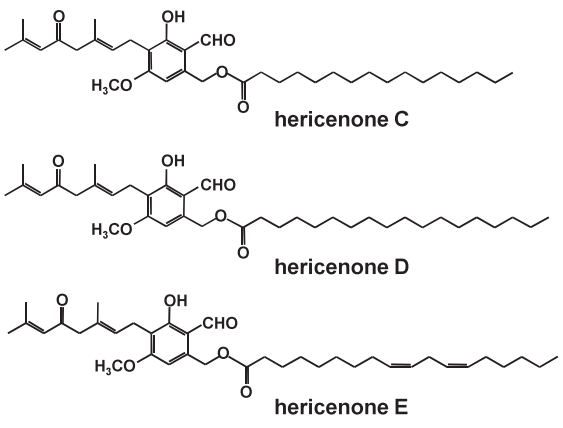

Fig. 3 Chemical structures of hericenone C, D and E.

lipid-soluble besides hericenones (19). In addition, the oral administration of *H. erinaceus* increased NGF mRNA expression in the mouse hippocampus (19). Therefore, we expect that the active compounds could be absorbed into blood and delivered into the central nervous system through the blood-brain barrier. Future studies will be required to further elucidate the active substances and the mechanism.

In conclusion, intracerebroventricular administration of Aβ(25-35) induced cognitive dysfunction resulting in a significant reduction of alternation behavior in the Y-maze test and the discrimination ratio in the novel-object recognition test. Importantly, *H. erinaceus* prevented the cognitive deficits induced by Aβ(25-35). These findings indicate that *H. erinaceus* may be a promising treatment for the prevention of cognitive dysfunction.

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