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Spatial Memory Impairment Without Apoptosis Induced by the Combination of Beta-Amyloid Oligomers and Cerebral Ischemia Is Related to Decreased Acetylcholine Release in Rats

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Abstract. The purpose of the present study was to examine the effect of beta-amyloid (Aβ) oligomers, not the fibrils that make up Aβ plaques, on spatial memory and the cholinergic system in rats. Recently, several researchers have suggested that small assemblies of Aβ, Aβ oligomers, caused memory loss during the early stages of Alzheimer's disease without showing cell death. In the present study, the combination of Aβ oligomers and cerebral ischemia, but not cerebral ischemia alone, significantly impaired spatial memory without apoptosis in the CA1 region of the hippocampus. Donepezil, an acetylcholinesterase inhibitor, ameliorated this memory impairment. Therefore we examined acetylcholine (ACh) release from the dorsal hippocampus. A microdialysis study showed that spontaneous release of ACh was not significantly decreased by the combination of Aβ oligomers and cerebral ischemia; however, high K^+-evoked ACh release was decreased. These results suggest that a combination of Aβ oligomers and cerebral ischemia induces memory impairment by cholinergic synapse dysfunction without apoptosis. This model may be useful for developing new drugs for the treatment of early-phase Alzheimer's disease.

Keywords: Alzheimer's disease, oligomer, acetylcholine, memory impairment, behavior

Introduction

Alzheimer's disease (AD) is progressive dementia with senile plaques composed of beta-amyloid (Aβ). Aggregation of Aβ proceeds through several conformational states, including dimers, spherical oligomers, and strings of oligomers (protofibrils), before finally forming Aβ fibrils (1, 2). Neurotoxicity of Aβ requires peptide aggregation (3 – 5). In previous studies, many researchers have investigated the neurotoxicity of Aβ fibrils (6, 7) because Aβ fibrils are found in the brains of AD patients postmortem. Indeed, Aβ fibrils caused neuronal cell death both in vitro and in vivo and impaired spatial memory in rats (7). However, fibrils are not thought to be the exclusive cause of AD because Aβ plaques do not predict the severity of dementia in AD patients (8). In amyloid precursor protein (APP) transgenic mice that overexpress mutant APP and produce a large amount of Aβ, cognitive deterioration precedes before Aβ plaques show clearly and occurs without neuron loss (9 – 12). Memory loss in early-stage of AD patients and APP transgenic mice may not be attributable to Aβ fibrils. Rather, small aggregations of Aβ such as Aβ oligomers may cause memory loss before Aβ finally accumulates as Aβ fibrils in the brain (12). A newer hypothesis has emerged in which early memory loss is related to a synapse dysfunction caused by Aβ oligomers (13). Furthermore early memory loss may be considered to be independent of neuronal cell death.

Some researchers have successfully produced Aβ oligomers synthetically. Aggregation of Aβ is dependent on incubation time, and synthetic Aβ oligomers are

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formed with shorter incubation times compared to Aβ fibrils. Synthetic Aβ oligomers form a globular body with diameters of 2 – 5 nm or a small string of 4 – 8 nm (14 – 16). The globular forms of Aβ oligomers prepared from Aβ 40 have weaker neurotoxicity than fibrils in Neuro-2A neuroblastoma cells (15). Natural, cell-derived Aβ oligomers inhibit hippocampal long-term potentiation in vivo and impair cognitive function in vivo (17 – 19). However, there are no reports that examined the effect of Aβ oligomers on ACh release related to memory in vivo.

We previously demonstrated that the combination of intracerebroventricular (i.c.v.) administration of Aβ aggregates and transient cerebral ischemia significantly impaired spatial memory linked to apoptosis in the pyramidal neurons of the CA1 region of the hippocampus in rats (20). The reasons why we combined Aβ with cerebral ischemia are 1) clinical reports indicate that AD patients with brain infarcts had poorer cognitive function than those without infarcts (21), suggesting that Alzheimer's patients with a history of cerebrovascular disease have a more rapid progression of dementia and 2) hypoxia treatment enhances Aβ-induced apoptosis in cultured hippocampal neurons (22).

In the present study we examined whether the combination of Aβ oligomers and cerebral ischemia would impair spatial memory and whether this combination would induce apoptosis in the pyramidal neurons of the CA1 region of the hippocampus. ACh release was also measured by microdialysis in the dorsal hippocampus of free moving rats in order to determine whether the combination of Aβ oligomers and cerebral ischemia would induce cholinergic dysfunction. We also examined the effect of donepezil, an acetylcholine inhibitor used in therapy for AD, on the spatial memory impairment, in order to confirm relationship of the cholinergic dysfunction and the spatial memory impairment induced by the combination of Aβ oligomers and cerebral ischemia.

Materials and Methods

Animals

The experiments were performed on male Wistar rats weighing 230 – 270 g (Kyudo, Saga). Rats were housed in groups of 4 – 5 per cage (30 × 35 × 17 cm) in a room with controlled temperature (23 ± 2°C), relative humidity of 60 ± 2%, and a 12-h light/dark cycle with the light period starting at 7:00 am. Food and water were available ad libitum except during the restricted feeding schedule. Experiments were carried out in compliance with the guidelines stipulated by the Animal Care and Use Committee of Fukuoka University.

Aβ oligomers

Aβ oligomers were prepared following a modification of Yoshiike's procedure (16). Aβ 40 and Aβ 42 peptides were purchased from Anaspec, Inc. (San Jose, CA, USA). A stock solution (stock 1) of Aβ was prepared by dissolving powdered Aβ peptide in 100% 1,1,1,3,3,3,-hexafluoro-2-propanol (HFIP) to a final concentration of 1 mg/ml. After shaking for 2 h at 4°C, the Aβ stock 1 solution was aliquoted into tubes. Aliquots of Aβ 40 and Aβ 42 were redissolved in 50% HFIP / 14% NH3 solution and 100% HFIP, respectively; and then this Aβ stock 2 solution was aliquoted into tubes and vacuum-dried. Just prior to each experiment, they were dissolved in a HEPES-buffered solution to a final concentration of 0.045 μg/μl. Then, they were mixed at a ratio of Aβ 40 : Aβ 42 = 10:1 and incubated at 37°C for 150 min.

Eight-arm radial maze task

The apparatus and procedure were as previously described (20). In pre-training, rats were habituated in groups of 5 rats to the apparatus and the reinforcer food pellets daily (each session 10 min, repeated 3 times at intervals of 60 min) for 3 days before training. In each training session, the rat was placed within a circular plastic cage on the platform in the middle of the 8-arm radial maze. After 1 min, the cage was lifted, and the rat was allowed to move freely in the maze. The trial continued until the rat had either entered all 8 arms or 10 min had elapsed. Rats that proceeded through the maze using non-spatial strategies, that is, repeatedly choosing the arm adjacent to (45°) or the arm that was 3 arms away from (135°) the one currently visited, were excluded from the present experiment because they were considered to not have acquired spatial memory. The following parameters were considered the criteria for maze performance: 1) the number of correct choices (CC) in the initial 8 chosen arms (entry into an arm that the animal had not previously visited), and 2) the number of errors, EC (reentry into an arm that the rat had previously visited). The training was performed once/day for more than 3 weeks. Only the rats that made no errors or only one error for 3 consecutive days were selected for the study. The test was performed at day 7 after cerebral ischemia or 1 h after the last Aβ oligomers injection. Maze performance was observed using a Video Image Motion Analyzer (AXIS30; Neuroscience Co., Tokyo).

Four-vessel transient cerebral ischemia

Four-vessel occlusion was performed according to the method previously described by Iwasaki et al. (20). Briefly, rats were anesthetized with 50 mg/kg, i.p.

sodium pentobarbital (Tokyo Kasei, Tokyo) and immobilized in a stereotaxic apparatus. The bilateral vertebral arteries were electrocauterized using a bipolar coagulator (MICRO-3D; Mizuho Industrial Co., Tokyo). The bilateral common carotid arteries were then exposed and threaded. The next day, the common carotid arteries were compressed by clips, and cerebral circulation was interrupted for 10 min. Rats that did not exhibit a loss of their righting reflex during arterial occlusion were excluded from subsequent experiments.

Stereotaxic procedure

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame (Narishige Scientific Instruments, Tokyo). Guide cannulae were implanted according to Paxinos and Watson (23). In microdialysis studies, the guide cannulae (0.85-mm o.d., 0.75-mm i.d., and 13 mm in length; Eicom Kyoto) were implanted into the dorsal hippocampus at the following coordinates: anteroposterior (AP) = –4.0 mm posterior to bregma; lateral (L) = 3.3 mm from the midsagittal line; and dorsoventral (DV) = 1.8 mm relative to the skull surface. For i.c.v infusion, guide cannulae (0.71 ± 0.02-mm o.d., 0.41 ± 0.02-mm i.d., and 13 mm in length) were implanted bilaterally into the lateral cerebral ventricles at AP = –0.8 mm, L = ± 1.3 mm, and H = 3.3 mm. The implanted cannulae were anchored by 2 screws driven into the skull, fixed by dental acrylic cement, and then secured with dummy cannulae kept in place by a cap. After surgery, each rat was injected with penicillin in the hindquarter muscle (100,000 U) and individually housed after the operation.

Microdialysis procedures

The extracellular ACh levels were measured in the dorsal hippocampus by microdialysis, 3 days after surgery, in unanesthetized freely moving rats. The measurements were performed at the same time point as behavioral testing. The microdialysis probes were 13-mm-long, 0.5-mm i.d., and 0.6-mm o.d. (Eicom), with an active dialysis membrane exposed at the tip. The dialysis membrane was a U-shaped-tube (each arm 2-mm-long, 0.22-mm i.d., 3.14-mm o.d., total length of both arms 4 – 5 mm) made of cellulose hollow fiber with a molecular weight cut-off value of 50 kDa. Ringer-primed probes were implanted through the guide cannulae with the U-shaped dialysis membrane parallel to the longitudinal axis of the brain, protruding 2 mm into the dorsal hippocampus through the tip of the guide cannulae, and were secured by caps. Rats were then placed in a Plexiglas chamber (50 cm × 35 cm × 35 cm). The inlet of the probe was connected to the descending limb of the spatial 2-way tubing that was, in turn, connected via a pair-ring swivel system to another Teflon tube connected to a micro syringe driven by a pump (Eicom SP-64, Eicom). The outlet of the probe was connected to ascending limb of the swivel system, connected by another Teflon tube to an autoinjector (Eicom-EAS-20). The tubes were 0.4-mm o.d., 0.1-mm i.d., and 50-cm-long (Eicom). The implanted probe was perfused with Ringer's solution or high potassium Ringer's solution (147 mM NaCl, 4.02 mM KCl or 100 mM KCl for high K^+, and 2.2 mM $CaCl_2$:$2H_2O$) containing 0.1 mM eserine, at a flow rate of 1 μl/min. Dialysate aliquots of 20 μl were collected every 20 min.

Determination of extracellular ACh levels

The levels of ACh in the dialysates were assayed by directly injecting the dialysates into a high-performance liquid chromatography apparatus (HPLC, Eicom EP-300 Liquid chromatography) by an autoinjector kept at room temperature. ACh was separated using an analytical reverse-phase column (Eicompak AC-GEL, 2.0-mm i.d., 150 mm; Eicom). A guard (pre-) column (3.0-mm i.d., 4.0 mm with CH-GEL filter; Eicom) was placed before the analytical column. An enzyme column (AC-ENZYMPAK, 3.0-mm i.d.; Eicom), in which acetylcholinesterase and choline oxidase are immobilized on controlled pore glass (56.4-nm pore diameter, 200/400 mesh glass beads), was placed after the analytical column. Following separation on the analytical column, ACh was hydrolyzed by acetylcholinesterase to acetate and choline in a post-column enzyme reactor, and the choline was oxidized by choline oxidase to produce betaine and hydrogen peroxide. The analytical and enzyme columns were maintained at 33°C by a column oven (Eicom ATC-300). Detection was performed using an electrochemical detector (Eicom ECD-300) with a platinum electrode (WE-PT) set at +450 mV *vs* Ag/AgCl reference electrode (Ag/AgCl RE-100). The mobile phase (50 mM Na_2HPO_4:$12H_2O$, 50 mM H_3PO_4, 1.23 mM sodium 1-decanesulfonate, and 0.013 mM EDTA:2Na, pH 8.2) was degassed (Eicom DG-300) and pumped at a flow rate 0.15 ml/min. An internal standard (isopropylhemicholine, 0.05 pmol/μl) was injected into the autoinjector at a rate of 1 μl/min, simultaneously with the perfusion Ringer solution.

Peaks were recorded using a Powerchrom integrator (Eicom). To evaluate the amount of ACh in the samples, a linear regression curve was constructed using standards (1 pmol) of choline, isopropylhomocholine, and ACh. Peak heights in the samples being analyzed were compared to standards by means of a data processor (Eicom EPC-300). The detection limit, based upon the signal-to-noise ratio, was 10 fmol

ACh. The HPLC standards ACh perchlorate and choline [(2-hydroxyethyl) trimethylammonium] were purchased from Sigma (St. Louis, MO, USA). Isopropylhomocholine was from Eicom.

After the stabilization period and establishment of a stable basal ACh level (for at least 2 h), 4 fractions were collected under basal conditions. Four fractions were then collected following a change of the high potassium Ringer's solution.

At the end of the experiment, rats were decapitated, their brains were quickly removed and cut into at 50-μm-thick sections, and placement of the microdialysis probes in the dorsal hippocampus was verified.

TUNEL staining

On the day behavioral test was finished, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused transcardially with heparinized saline followed by 4% paraformaldehyde. Brains were removed, fixed overnight in paraformaldehyde, and stored in paraffin embedding, before being cut into 10-μm sections. Sections were deparaffinized and dehydrated.

To detect apoptotic cells, each section was stained using the terminal deoxynucleotidyl transferase (TdT)-mediated fluorescein-deoxyuridinetriphosphate (dUTP) nick-end labeling (TUNEL) technique (TACS II; Trevigen, MD, USA). Propidium iodide (1 μg/ml, red, Sigma) was used to stain normal CA1 cells. Fluorescein-dUTP-labeled fragmented DNA can be visualized directly by fluorescence microscopy (Leica, DMRA, Germany). Sections including the CA1 field of the dorsal hippocampus were examined for the presence of TUNEL-positive cells (green, FITC). For the positive control, the section was treated with 2 mg/ml deoxyribonuclease I (DNase I, Sigma) for 10 min before staining.

Experimental paradigm and groups

In the Aβ oligomers group, Aβ oligomers were injected i.c.v. once daily. In the combination of Aβ oligomers and cerebral ischemia group, Aβ oligomers were injected daily for 7 days after cerebral ischemia (10 min). Aβ oligomers were administrated bilaterally (200 pmol/20 μl) using an injection cannula (0.35 ± 0.01-mm o.d., 0.17 ± 0.02-mm i.d., and 14 mm length) connected by PE-tubing (1.09-mm o.d., 0.38-mm i.d.; INTRAMEDIC; Becton Dickinson, MD, USA) to a perfusion pump (CMA/100; Microdialysis AB, Stockholm, Sweden) driven at rate of 1 μl/min. The injection cannulae protruded 1-mm beyond the guide cannulae.

Drug testing

Donepezil hydrochloride (referred to simply as donepezil) was obtained from Eisai, Tokyo. Donepezil was dissolved in distilled water and orally administered 60 min prior to the test.

Statistical analysis

The results of radial maze performance were evaluated for statistical significance using non-parametric analysis of variance (Kruskal-Wallis test) followed by Dunn's test for non-parametric multiple comparisons. ACh levels were analyzed using the Mann Whitney's U-test. The criterion for statistical significance was considered to be $P<0.05$. Values are expressed as means ± S.E.M.

Results

Effects of Aβ oligomers and cerebral ischemia on spatial memory

In the 8-arm radial maze task, no significant changes in performance were observed either with cerebral ischemia [correct choices (CC), 7.6 ± 0.2; error choices (EC), 0.4 ± 0.2] or Aβ oligomers alone (CC, 7.3 ± 0.2; EC, 1.3 ± 0.5), compared with the sham group (CC, 7.5 ± 0.1; EC, 0.8 ± 0.2) (Fig. 1). However, the combination of Aβ oligomers and cerebral ischemia significantly decreased the number of CC (5.5 ± 0.3) and tended to increase the number of EC (7.2 ± 1.6) compared with the sham group ($P<0.05$ for CC, Dunn's test) (Fig. 1). In addition, the combination of Aβ oligomers and cerebral ischemia significantly decreased the number of CC and tended to increase the number of EC compared to the cerebral ischemia alone group ($P<0.05$ for CC, Dunn's test) (Fig. 1). Compared with the Aβ oligomers alone group, the combined group showed a tendency toward a decrease in the number of CC and an increase in the number of EC.

The combination of Aβ oligomers and cerebral ischemia did not induce the expression of TUNEL-positive cells

On the last day of Aβ oligomers injection, the combination of Aβ oligomers and cerebral ischemia did not affect TUNEL staining (Fig. 2: A and B). We checked TUNEL-positive cells in a section treated with DNase I (2 mg/ml) as the positive control (data not shown).

Effect of the combination of Aβ oligomers and cerebral ischemia on ACh release from the dorsal hippocampus

Spontaneous release of ACh from the dorsal hippocampus had a tendency to decrease in rats receiving a combination of Aβ oligomers and cerebral ischemia

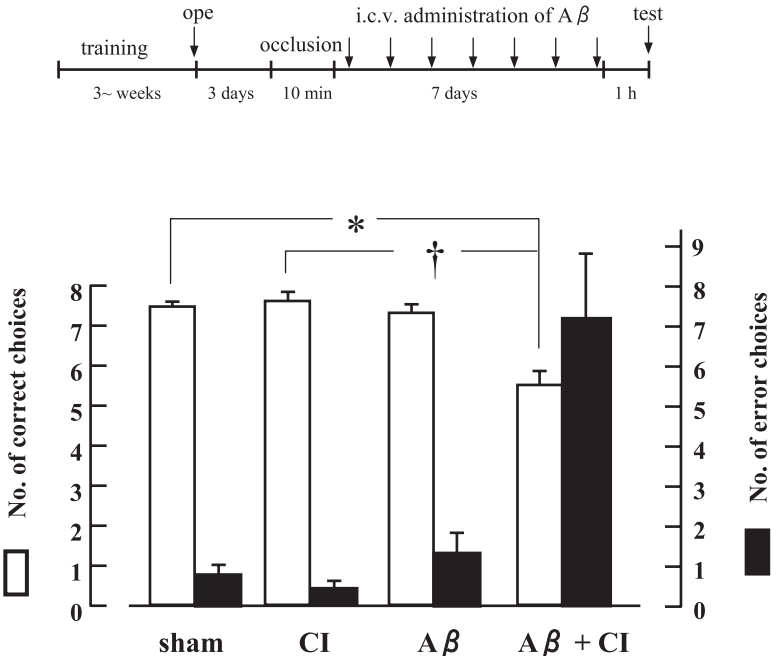

Fig. 1. Effect of Aβ oligomers and cerebral ischemia (CI) on spatial memory in the 8-arm radial maze. The test was performed at day 7 after cerebral ischemia or 1 h after the last Aβ oligomers injection. The number of correct choices is presented as empty bars and the number of error choices is presented as filled bars in sham-operated rats (n = 13) and rats receiving cerebral ischemia (n = 7), Aβ oligomers (n = 7), or Aβ oligomers + cerebral ischemia (n = 12). Values are expressed as means ± S.E.M. *P<0.05, $^{\dagger}P$<0.05 (Dunn's test)

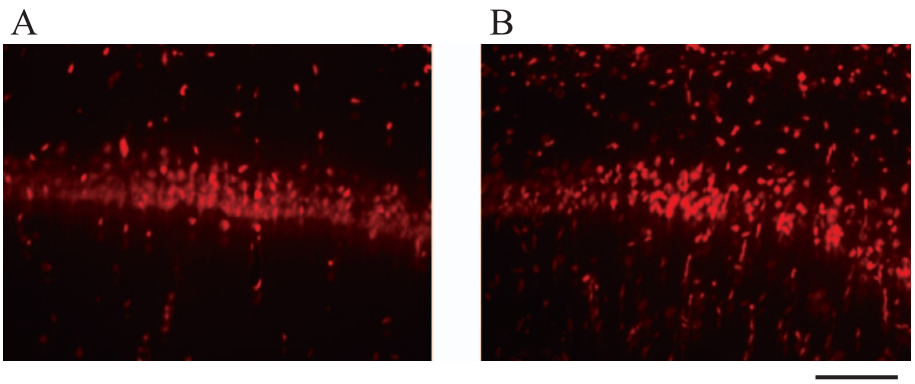

Fig. 2. TUNEL staining of rat hippocampal CA1 neurons. A and B: red cells: propidium iodide-stained normal CA1 cells; green cells: FITC-stained TUNEL-positive (apoptotic) cells. Representative photomicrographs are coronal sections of the CA1 field of the hippocampus from sham-operated rats (A) and rats receiving Aβ oligomers + cerebral ischemia (B). Scale bar = 100 μm

(107.5 ± 44.8 fmol/μl) compared with the sham group (180.2 ± 66.6 fmol/μl) (Fig. 3A), but this decrease was not significant. By contrast, rats receiving a combination of Aβ oligomers and cerebral ischemia showed a significant decrease in high K^+-evoked increase in ACh release (97.2 ± 5.3%, P<0.05, Mann-Whitney's U-test) compared with the sham group (1053 ± 358.8%, Fig. 3B).

Effect of donepezil on spatial memory impairment induced by the combination of Aβ oligomers and cerebral ischemia

Donepezil significantly improved the spatial memory impairment induced by the combination of Aβ oligomers and cerebral ischemia. At a dose of 3 mg/kg, the number of CC significantly increased, while that of EC showed a tendency to decrease (CC, 6.9 ± 0.3; EC, 2.1 ± 0.7; P<0.01 for CC, Mann-Whitney's U-test) compared to the non-treatment group (CC, 5.7 ± 0.3; EC, 6.3 ± 1.1) (Fig. 4).

Discussion

In this study, we found that the combination of Aβ oligomers and cerebral ischemia impairs spatial memory in rats without apoptosis of pyramidal neurons in the CA1 region of the hippocampus. This combination significantly decreased high-K^+ evoked ACh release

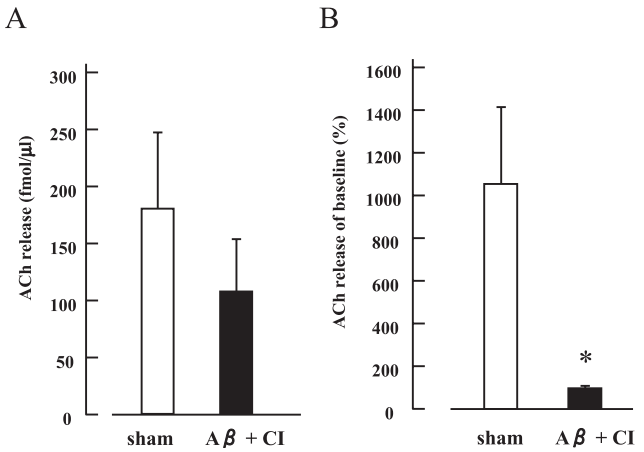

Fig. 3. Effect of Aβ oligomers and cerebral ischemia (CI) on ACh release in the dorsal hippocampus. Spontaneous ACh release (A) and high-potassium-evoked ACh release (B) in sham-operated rats (n = 4) and rats given Aβ oligomers + cerebral ischemia (n = 5). Extracellular ACh levels are expressed as fmol/μl (A) and as a percentage of the baseline concentration (B). Values are expressed as the mean ± S.E.M. **P*<0.05, compared to sham-operated rats (Mann-Whitney's U-test).

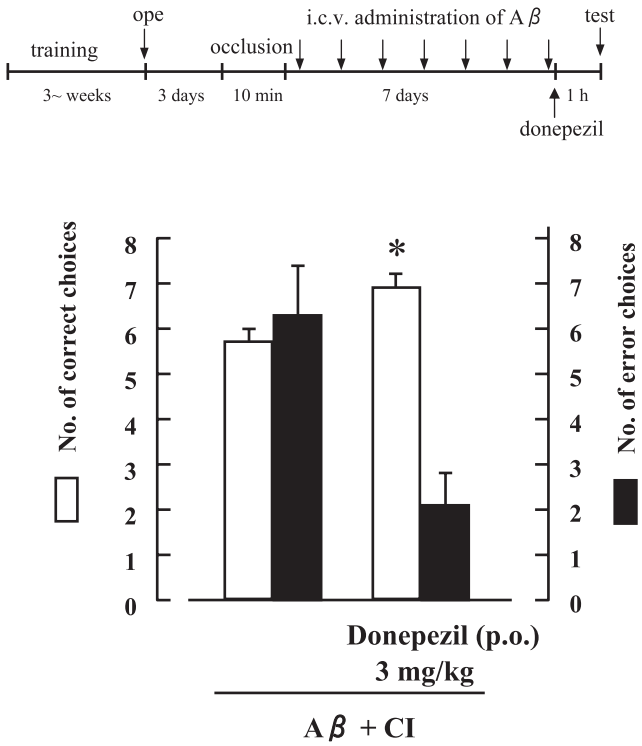

Fig. 4. Effect of donepezil on the spatial memory impairment induced by the combination of Aβ oligomers and cerebral ischemia (CI). The test was performed 1 h after the last Aβ oligomers injection. The numbers of correct choices are presented as empty bars and the numbers of error choices are presented as filled bars in rats receiving Aβ oligomers + cerebral ischemia with (n = 9) or without (n = 18) donepezil treatment. Donepezil was orally administered (peroral administration, p.o.) 60 min prior to the test. Values are expressed as means ± S.E.M. **P*<0.01, compared to the non-treatment group (Mann-Whitney's U-test).

from the dorsal hippocampus. These results suggest that the combination of Aβ oligomers and cerebral ischemia may impair spatial memory via synapse dysfunction of the cholinergic system, without inducing apoptosis. We previously observed that the combination of Aβ aggregates and cerebral ischemia impaired spatial memory with apoptosis and decreased high K^+-evoked ACh release in rats (20). Taken together, these results suggest that Aβ oligomers might relate more closely to synapse dysfunction of the cholinergic system than Aβ aggregates. The current results emphasize that the model generated by a combination of Aβ oligomers and cerebral ischemia might reflect early memory loss in AD patients and APP transgenic mice.

Administration of Aβ oligomers alone did not impair memory (Fig. 1). However, Aβ oligomers have been reported to impair cognitive function in vivo (19). This difference in toxicity may be due to differences in conformation, incubation conditions, or injection period. Cerebral ischemia alone also did not impair memory at 7 days after reperfusion (Fig. 1). There are two possible reasons to explain how the combination of Aβ oligomers and cerebral ischemia impairs spatial memory (Fig. 1). One reason is synapse dysfunction, such as changes in neurotransmitter release. Cerebral ischemia decreases ACh release without causing death of CAl pyramidal cells (24). Aβ oligomers localize to cell processes in AD patients (25) and may target synapses. The combination of Aβ oligomers and cerebral ischemia may synergistically induce synapse dysfunction leading to decreased ACh release. The other possible reason for the spatial memory impairments is glutamate toxicity leading to apoptosis. Cerebral ischemia increases glutamate release at an early period both in vivo and in vitro (26, 27). In vitro studies have demonstrated that Aβ enhances glutamate neurotoxicity, increases fragmentation of DNA, and decreases cell viability (28, 29). The combination of Aβ oligomers and cerebral ischemia may synergistically increase glutamate and induce apoptosis. These results suggest that the combination of Aβ oligomers and cerebral ischemia may cause spatial memory impairment by synapse dysfunction or apoptosis.

We previously reported that the expression of TUNEL-positive pyramidal neurons in the CA1 correlated significantly with an impairment of spatial memory in an 8-arm radial maze in rats subjected to repeated ischemia and a combination of Aβ aggregates with cerebral ischemia (20, 30). However, in the CA1 of rats treated with the combination of Aβ oligomers and cerebral ischemia, TUNEL-positive cells could not be

detected (Fig. 2). Learning and memory deficits without neuronal cell death were also reported to be induced by the administration of lipopolysaccharide into rat hippocampus (31). Thus, memory impairment is caused even without neuronal cell death. In APP transgenic mice, cognitive deterioration precedes the formation of significant Aβ plaque and occurs without neuronal cell death (9 – 12), suggesting that early memory loss may not be related to these phenomena. Therefore the spatial memory impairment is not likely to be the result of apoptosis. These results suggest that spatial memory impairment induced by the combination of Aβ oligomers and cerebral ischemia may reflect early memory loss in AD patients and APP transgenic mice.

The combination of Aβ oligomers and cerebral ischemia significantly decreased high K^+-evoked release of ACh compared with the sham-operated group (Fig. 3B). By contrast, spontaneous ACh release was not significantly different between the sham-operated group and the treated group (Fig. 3A). The mechanism of high K^+–evoked release is unclear. High K^+–evoked release may reflect stored neurotransmitter or the efficiency of synapse vesicle recycling in presynapses. Ischemia induces presynaptic defects because phosphorylation of synapsin-I, a synaptic vesicle protein, is decreased (32). Moreover, Aβ oligomers localize to cell processes (25). Aβ oligomers induce a decrement in dynamin 1, a protein that is essential for synaptic vesicle recycling, but do not decrease the levels of synaptophysin (33). Hence, Aβ oligomers induce synaptic dysfunction without presynaptic loss. Thus, both Aβ oligomers and ischemia target synaptic vesicle proteins required for synapse vesicle recycling. These results suggest that the combination of Aβ oligomers and cerebral ischemia may change protein levels, as described above, and induce synaptic dysfunction through the defects in synapse vesicle recycling. The treatment of Aβ oligomers or cerebral ischemia alone did not affect spatial memory (Fig. 1), suggesting that their treatments could not change ACh release significantly. Therefore we did not measure the effect of Aβ oligomers or cerebral ischemia alone on ACh release.

Donepezil, an acetylcholinesterase inhibitor, administered just before the test, ameliorated the spatial memory impairment in the 8-arm radial maze (Fig. 4). Donepezil increases extracellular ACh levels in the hippocampus in rats (34, 35). These results suggest that the combination of Aβ oligomers and cerebral ischemia impairs spatial memory via cholinergic dysfunction and that activation of the cholinergic system by donepezil improves spatial memory impairment. We previously demonstrated that nilvadipine, a voltage-dependent Ca^{2+}-channel (VDCC) blocker, inhibited apoptosis and improved the spatial memory impairment induced by the combination of Aβ aggregates and cerebral ischemia (36). Nilvadipine may prevent apoptosis by blocking Ca^{2+} influx through VDCC and finally maintain spatial memory. However, nilvadipine did not ameliorate the spatial memory impairment induced by the combination of Aβ oligomers and cerebral ischemia (data not shown), strengthening the idea that the detrimental effect of the combination of Aβ oligomers and cerebral ischemia does not involve apoptosis. Indeed, Kelly also reported that a VDCC blocker did not prevent synapse dysfunction induced by Aβ oligomers (37). These results suggest that oligomer forms of Aβ impair memory by inducing cholinergic dysfunction without apoptosis.

In conclusion, the combination of Aβ oligomers and cerebral ischemia impairs spatial memory via cholinergic dysfunction without inducing apoptosis. This finding suggests that oligomer forms of Aβ may be involved in early memory loss related to cholinergic dysfunction during a period of no neuronal cell loss. This model may be useful for developing new drugs to treat the early phase of Alzheimer's disease.

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