e.g. [Leu] enkephalin enhances and impairs the acquisition of a one-way step-through active avoidance response in a dose-dependent manner [24,25]. To exclude a possible involvement of enkephalins, of which metabolism may be affected by the inhibition of neprilysin, in memory learning tasks in the rat, we administered naloxone (1 mg/kg, i.p.) to thiorphan-treated rats before each experiment. However, naloxone treatment had no effect on cognitive performance in the thiorphan-infused rats. The genetic approach supports our finding, because neprilysin deficiency does not significantly elevate enkephalin levels in the brain [47]. Thus, the cognitive impairment in the thiorphan-infused rats is not due to the presence of redundant enkephalin.

Previous reports have shown that continuous infusion of Aβ40 into the rat cerebral ventricle impairs several learning and memory tasks [33,34,49,52,53] and nicotine-stimulated extracellular ACh release [17,50]. In the present study, however, the rats continuously infused with thiorphan showed only an impairment of novelty discrimination in a configural version of object recognition behavior, and spatial memory in a probe trial of the water maze task, but no difference in the nicotine-stimulated ACh release, compared to the vehicle-treated rats. Such an inconsistency in the results obtained from between Aβ40- and thiorphan infusion model may be due to differences in the regions, where Aβ40 is accumulated and involved in cognitive functions; a significant accumulation of Aβ is observed in the cerebral cortex and hippocampus of Aβ40-infused rats, whereas only in the cerebral cortex of the thiorphan-infused rats. Regarding a region-specific accumulation of Aβ, Newell et al. [32] also reported that the intracerebroventricular infusion of thiorphan elevates only cerebral Aβ40 level in the rabbits, consistent with our observation. Therefore, this inconsistency may be explained by a region-specific Aβ40 accumulation derived from the distribution of thiorphan infused into the cerebral ventricle and different neuronal sensibility for the accumulation between the cerebral cortex and hippocampus. Furthermore, for Aβ40 infusion experiments human-type Aβ is generally used, whereas in the thiorphan infusion experiments accumulation of endogenous rodent-type Aβ is expected. As another possibility, it may be pointed out a difference of Aβ sequence between human and rodents. Because human-type Aβ shows higher self-assembly than rodent-type Aβ [4,38], Aβ accumulated by the Aβ40 infusion and thiorphan infusion may present different status for oligomerization and fibrilization each other.

Some cortex regions are necessary for the acquisition and retention of hippocampus-dependent memory such as the performance of object recognition task and Morris water maze task, and such cognition is disrupted by cortical lesions [3,12,57]. Our results suggest that the cortex played a critical role in novelty discrimination in configural version of object recognition behavior and spatial memory in a probe trial of water maze tasks. However, we need to further investigate neurochemically and neuropharmacologically whether the cerebral accumulation of Aβ induces dysfunctions of the cortex in the thiorphan-infused rats. In addition, Iwata et al. [21] have reported that direct infusion of thiorphan into the hippocampus of rats elevates the hippocampal Aβ levels. To investigate a role of neprilysin in the metabolism of Aβ and cognitive function in the hippocampus, we need to employ this model.

In conclusion, we demonstrated that the inhibition of endogenous neprilysin activity in vivo by intracerebroventricular infusion of thiorphan increases concentration of Aβ in the cortex and impairs some cognitive functions. Recently, some gene analyses have disclosed that single nucleotide or dinucleotide-repeated polymorphisms on the neprilysin gene increase susceptibility to AD [2,16,46]. These studies suggest not only that the polymorphisms of neprilysin gene could be a risk factor for the development of AD, and but also that the loss of brain neprilysin activity could be a pathogenic mechanism leading to the age-related deposition of Aβ and development of AD.

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Research report

Effects of memantine and donepezil on amyloid β-induced memory impairment in a delayed-matching to position task in rats

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Abstract

We investigated the effects of memantine and donepezil on amyloid β (Aβ)-induced memory impairment in rats, which was assessed by a delayed-matching to position (DMPT) paradigm in three-lever operant chambers. Aggregated Aβ1-40 was microinjected bilaterally (1 nmol/side) into both CA1 and CA3 subfields of the hippocampus in rats that had previously performed the DMTP task. Memantine (20 mg/(kg day), s.c.) was continuously infused by an osmotic minipump for 4 weeks from 3 days before the microinjection of Aβ. Donepezil (2.5 mg/kg, p.o.) was administered 60 min before the DMTP test session. Bilateral microinjections of Aβ1-40 into the hippocampus resulted in a delayed, but persistent impairment of DMTP performance, which appeared more than 50 days after the injection. Memantine prevented the development of Aβ-induced memory impairment, while donepezil symptomatically alleviated the deficits. Because of a ceiling effect, the combination of donepezil with memantine failed to produce any additive or synergic effects. These results support the clinical data showing that memantine and donepezil are effective for the treatment of Alzheimer's disease. Moreover, it is suggested that memantine is effective for preventing Aβ-induced short-term memory impairment.

Keywords: Memantine; Donepezil; Alzheimer's disease; Amyloid β; Working memory; Hippocampus; Cholinesterase inhibitor; NMDA receptor

1. Introduction

Alzheimer's disease (AD) is the most common cause of the progressive decline of cognitive function in aged humans, and is characterized by the presence of numerous senile plaques and neurofibrillary tangles accompanied by neuronal loss. Although the exact pathogenesis of neuronal degeneration and cognitive impairment in AD remains to be fully defined, several pharmacological strategies have been proposed for the treatment of the disease [39].

Because a remarkable dysfunction of the cholinergic system is present in the brains of AD patients, and has been shown to be correlated with the severity of the cognitive impairment [28], it has been proposed that enhancement of cholinergic neurotransmission may ameliorate cognitive impairment in AD [3]. In fact, cholinesterase inhibitors, including tacrine, donepezil, rivastigmine and galantamine, have been successfully developed and approved for the treatment of moderate to severe AD [16]. Donepezil, a potent and selective inhibitor of brain cholinesterase [15], showed encouraging results in palliative therapy for AD [32].

The other hypothesis regarding the mechanism of neurodegeneration in AD is that excessive activation of glutamate receptors might be responsible for part of the

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neuronal damage observed in AD [9,27,38]. Although it is unlikely that glutamate-mediated exicitotoxicity is the primary etiopathological factor in AD, it may significantly contribute to the development of neurodegeneration in AD and it also has been suggested that impaired glutamatergic neurotransmission (overactivation of *N*-methyl-D-aspartate (NMDA) receptors) plays a role in the cognitive deficit [4,25]. In fact, recent clinical studies have demonstrated that memantine, a moderate affinity and uncompetitive NMDA receptor antagonist [25], is effective for the treatment of AD [30,35]. In preclinical studies, apart from experiments showing improvement of learning, it has been demonstrated that memantine protects against neuronal and behavioral deficits in rats treated with quinolinic acid [21,44] or ibotenic acid [1]. At present, memantine has been approved in Europe and USA for the treatment of moderate to severe AD. According to the excitotoxic hypothesis in chronic neurodegenerative diseases including AD, it should be emphasized that memantine as a preventive therapy may be more effective in early stage AD. Therefore, the neuroprotective effects of memantine should be investigated in an animal model of early stage AD.

The senile plaques are composed of amyloid β (Aβ), a 39–43 amino acid peptide fragment of the amyloid β precursor protein [33]. Aβ is cytotoxic to neurons [43] and renders neurons vulnerable to various insults including excitotoxicity [18,39]. The amyloid cascade hypothesis has been proposed in the etiopathology of AD [10], and accumulating evidence supports the hypothesis [33,39]. We have previously demonstrated that a continuous intracerebroventricular infusion of Aβ1-40 or Aβ1-42, but not Aβ40-1, causes learning and memory impairment, which was accompanied by cholinergic dysfunction [12,23,40], overproduction of nitric oxide [36,37] and oxidative stress [14,41]. The Aβ-induced memory impairment was exaggerated by ovariectomy [42], but ameliorated by antioxidants such as α-tocopherol [41] and inducible NO synthase inhibitors [36,37]. Acute injections of Aβ into the cortex or hippocampus also produce neurodegeneration and memory impairment in rodents [2,8,20,24,31], although the results are somewhat controversial. The Aβ-induced learning and memory impairment is a valuable model to assess the effects of novel antidementia drugs [39].

In the present study, we examined the effects of memantine on Aβ-induced memory impairment in rats and compared them with those of donepezil. We used a delayed-matching to position (DMPT) paradigm in three-lever operant chambers to investigate short-term memory [5,6,22], the cognitive domain being impaired in early stage AD [11,17].

2. Materials and methods

2.1. Animals

Male Fischer 344 rats, 11–12 weeks old and weighing 230–260 g at the start of experiment, were obtained from Charles River Japan (Yokohama, Japan). The animals were housed in plastic cages and kept in a regulated environment (23 ± 1 °C, 50 ± 5% humidity), with a 12-h light:12-h dark cycle (lights on at 9:00 a.m.). All animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Kanazawa University.

2.2. DMTP task

The animals were subjected to a food deprivation regime that reduces body weight to 75–80% of the initial weight for 7 days before the start of training. The operant chambers used and the training and test procedures for the DMTP task in the present study were the same as previously described by Miyamoto et al. [20]. Two operant chambers (San Diego Instruments, San Diego, CA, USA) were used, and each chamber had three retractable levers and three panel lights (4 cm × 4 cm) for sample stimulation above the retractable levers. The feeder connected to the food dispenser was located below the center lever, and a house light was located above the center panel light.

In the DMTP task, one of the three panel lights was illuminated for 5 s, and then the three levers were presented into the box, one of four delays (0, 8, 16, 32 or 64 s) being randomly enforced between the time the panel light was turned off and the presentation of the three levels. The sequence of the position of the panel light illuminated was randomly selected. If the rat pressed the correct lever, which was located just below the lit panel light, the rat was rewarded with a food pellet (45 mg) and all the levers were retracted. The next trial started 30 s after food reinforcement. If the rat failed to press the correct lever within 10 s or pressed one of the wrong levers, the levers were withdrawn with no reinforcement, and there was an intertrial interval of 30 s before the onset of the next trial. Each daily session consisted of 54 trials with four delays: 18 trials for 0-s delay, 12 trials for 8-s delay, 9 trials for 16-s delay, 9 trials for 32-s delay and 6 trials for 64-s delay presented randomly. The position of the correct lever for each delay was balanced within each session and presented in a different random order each day. The correct response percentage [(correct responses/total responses) × 100] on each delay was calculated. The number of trials completed (lever pressing within 10 s of the presentation of the three levers) was also recorded, and the percentage of trials completed [(trials completed/total trials) × 100] was calculated. Eleven rats whose correct response rate was more than 80% at the 0-s delay for 2 consecutive days were used for the microinjection of Aβ1-40.

2.3. Microinjection of Aβ1-40 into the hippocampus

Aβ1-40 (Bachem, Feinchemikalien AG, Switzerland) was dissolved in distilled water at a concentration of 1 nmol/μl, and incubated at 37 °C for 7 days to promote the aggregation [29]. The rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and restrained in a stereotaxis apparatus. Eight rats received bilateral microinjections of aggregated Aβ1-40 (injection volume: 1 μl/site) into both CA1 (A: −4.3, L: ±2.0, V: 2.6) and CA3 (A: −3.3, L: ±2.6, V: 3.5) subfields of the hippocampus, according to the atlas of Paxinos and Watson [26]. The vehicle (distilled water) was microinjected bilaterally into the hippocampal CA1 and CA3 in control rats (n=3).

2.4. Measurement of locomotor activity

Each rat was placed in a standard transparent rectangular cage (50 cm × 50 cm × 50 cm high), and the locomotor activity was measured for a period of 30 or 60 min using an infrared detector (Neuroscience, Tokyo, Japan) placed over the cage.

2.5. Drug treatment and experimental design

Memantine HCL (Batch: R8825) and donepezil (Lot: 13031705) were kindly donated by Merz Pharmaceuticals (Frankfurt, Germany) and Eisai Co. Ltd. (Tsukuba, Japan), respectively. Memantine was dissolved in saline, and was continuously infused s.c. at a dose of 20 mg/(kg day) with an Alzet osmotic pump (model 2ML2, Alza, Palo Alto, CA) for 4 weeks from 3 days before to 25 days after the intrahippocampal injections of Aβ1-40 ($n=4$). The dose of memantine (20 mg/(kg day)) used in the present study has been reported to yield pseudo steady-state serum levels close to the therapeutic range (1.2 μM) [21]. Some Aβ-treated rats ($n=4$) and control rats ($n=3$) received implantations of water-filled osmotic pumps. The osmotic pumps were renewed on day 11 to maintain the infusion of memantine or the vehicle until day 25. Accordingly, three groups with different treatments were prepared: control ($n=3$), Aβ-vehicle ($n=4$) and Aβ-memantine groups ($n=4$).

Donepezil was dissolved in distilled water and administered p.o. at a dose of 2.5 mg/kg. The dose of donepezil in the present study was selected based on our preliminary studies: donepezil at 2.5 mg/kg significantly ameliorated scopolamine (0.1 mg/kg)-induced impairment of DMTP performance, but had no effect on the performance in control animals (unpublished observations).

The experimental schedule is shown in Fig. 1. On days −1, 7, 9, 16, 23, 30, 37, 44, 51, 58, 65 and 72 after Aβ infusion, all animals in three groups were administered distilled water 1 h before the test session of the DMTP task. On the next day (days 10, 17, 24, 31, 38, 45, 52, 59, 66 and 73), they were administered donepezil 1 h before the test session. Locomotor activity of animals was measured on day 79 or 80 after Aβ infusion.

2.6. Histology

On day 82, the rats were anesthetized with pentobarbital and transcardially perfused with 250 ml of heparinized (0.1%, v/v) saline followed by 250 ml of phosphate-buffered saline (pH 7.4) containing 4% paraformaldehyde. Brains were removed and post-fixed in the same fixative overnight. After being cryoprotected with 30% sucrose, brains were cut into 40-μm-thick coronal sections. The sections throughout the hippocampus were mounted on gelatin-coated slides and stained with cresyl violet.

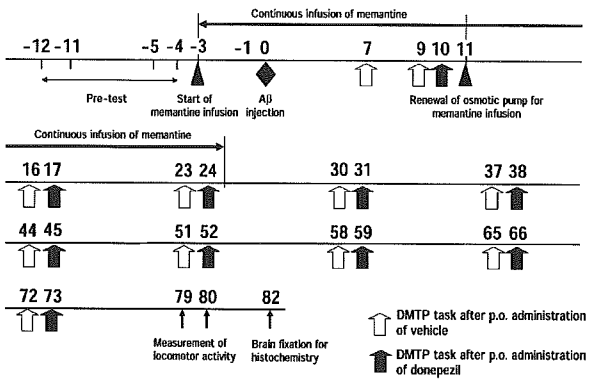

Fig. 1. Experimental schedule.

2.7. Statistical analysis

All data were expressed as the mean ± S.E. Statistical significance was determined with a one-way or two-way analysis of variance (ANOVA, repetitive measures), followed by the Student–Newman–Keuls test for multi-group comparisons when F-ratios were significant ($P<0.05$).

3. Results

Bilateral injections of Aβ1-40 into both CA1 and CA3 subfields of the hippocampus in rats that had previously performed the DMTP task had little acute effect on DMTP performance. Moreover, continuous infusion of memantine had no effect on DMTP performance in Aβ-treated rats. Fig. 2 illustrates the changes in DMTP performance at the 0-s (A and B) and 8-s delay (C and D) in Aβ-injected rats with or without memantine treatment. Aβ1-40 and memantine had little effect on either the percentage of correct responses (Fig. 2A for 0-s delay: $F(2,8)=0.187$, $P>0.05$; Fig. 2C for 8-s delay: $F(2,8)=0.424$, $P>0.05$) or response time (Fig. 2B for 0-s delay: $F(2,8)=0.486$, $P>0.05$; Fig. 2D for 8-s delay: $F(2,8)=0.646$, $P>0.05$) by day 44 after Aβ1-40 injections. As shown in Fig. 3, Aβ1-40 had no effects on average DMTP performance from days 7 to 44. Moreover, continuous infusion of memantine for 4 weeks (from 3 days before to 25 days after the Aβ1-40 injections) did not affect the DMTP performance of Aβ1-40-treated rats. A two-way ANOVA revealed a significant effect of delay (Fig. 3A for choice accuracy: $F(4,296)=97.667$, $P<0.0001$; Fig. 3B for response time: $F(4,296)=5.348$, $P<0.001$), but not group (Fig. 3A for choice accuracy: $F(2,74)=1.854$, $P>0.05$; Fig. 3B for response time: $F(2,74)=2.878$, $P>0.05$).

On day 51 and thereafter, significant changes in DMTP performance were observed. A one-way ANOVA with repeated measures revealed a significant effect of group at the 0-s (Fig. 2A for choice accuracy: $F(2,8)=0.082$, $P>0.05$; Fig. 2B for response time: $F(2,8)=6.817$, $P<0.05$) and 8-s delay (Fig. 2C for choice accuracy: $F(2,8)=14.180$, $P<0.01$; Fig. 2D for response time: $F(2,8)=10.345$, $P<0.01$). Fig. 4 shows the average DMTP performance from days 51 to 72 following Aβ1-40 injections into the hippocampus. A two-way ANOVA revealed significant effects of delay (Fig. 4A for choice accuracy: $F(4,164)=36.750$, $P<0.0001$; Fig. 4B for response time: $F(4,164)=4.737$, $P<0.01$) and group (Fig. 4A for choice accuracy: $F(2,41)=9.448$, $P<0.001$; Fig. 4B for response time: $F(4,41)=9.012$, $P<0.001$), but not delay × group interaction (Fig. 4A for choice accuracy: $F(8,164)=1.225$, $P>0.05$; Fig. 4B for response time:

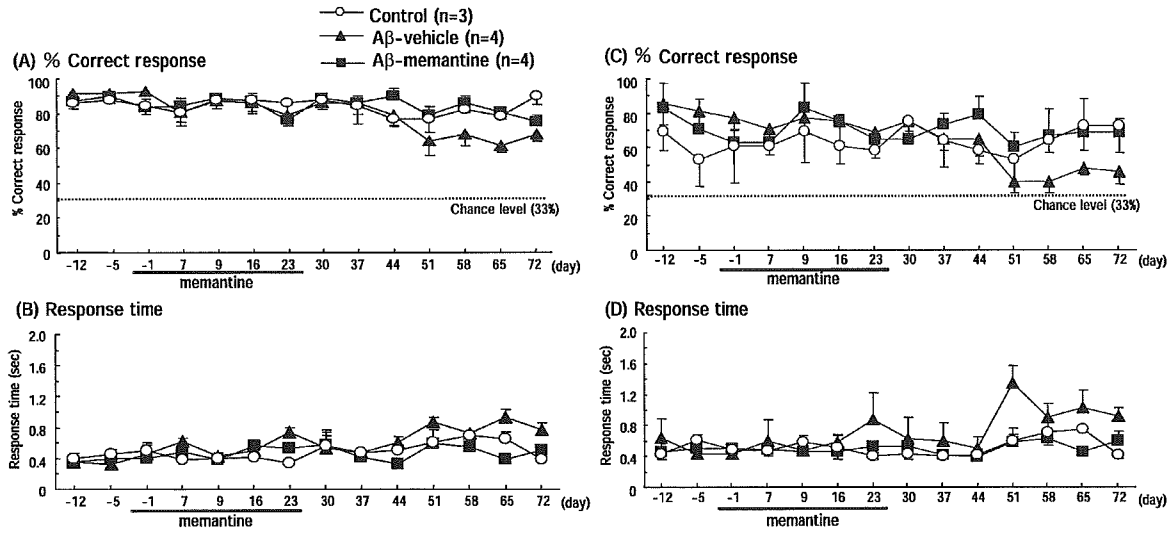

Fig. 2. Changes in DMTP performance at the 0-s (A and B) and 8-s delay (C and D) following bilateral microinjections of Aβ1-40 into the hippocampus in control, Aβ-vehicle and Aβ-memantine groups. Memantine (20 mg/(kg day)) was continuously infused with the Alzet osmotic pump for 4 weeks from 3 days before to 25 days after Aβ1-40 infusion. All animals were administered distilled water 1 h before the test sessions. (A and C) Percent correct response and (B and D) response time. Values indicate the mean ± S.E. ($n=3$ for control group, $n=4$ for Aβ-vehicle and Aβ-memantine groups).

$F(8,164)=1.443$, $P>0.05$). A post hoc analysis with the Student–Newman–Keuls test revealed that Aβ-vehicle group showed a significant decrease in choice accuracy ($P<0.05$) and an increase in response time ($P<0.05$) compared with the control group. Memantine significantly prevented the Aβ-induced decrease in choice accuracy ($P<0.05$) and increase in response time ($P<0.05$).

Fig. 5 shows the DMTP performance when the animals in the control, Aβ-vehicle and Aβ-memantine groups were administered donepezil (2.5 mg/kg, p.o.) 1 h before

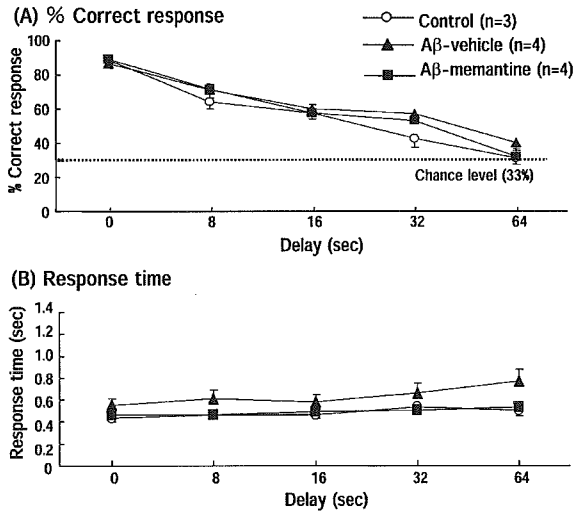

Fig. 3. Average DMTP performance from days 7 to 44 after bilateral microinjections of Aβ1-40 into the hippocampus in control, Aβ-vehicle and Aβ-memantine groups. (A) Percent correct response and (B) response time. Memantine (20 mg/(kg day)) was continuously infused with the Alzet osmotic pump for 4 weeks from 3 days before to 25 days after Aβ1-40 infusion. Values indicate the mean ± S.E. ($n=3$ for control group, $n=4$ for Aβ-vehicle and Aβ-memantine groups). A two-way ANOVA revealed no significant effect of group on the percent correct response [$F(2,74)=1.854$, $P>0.05$] or response time [$F(2,74)=2.878$, $P>0.05$].

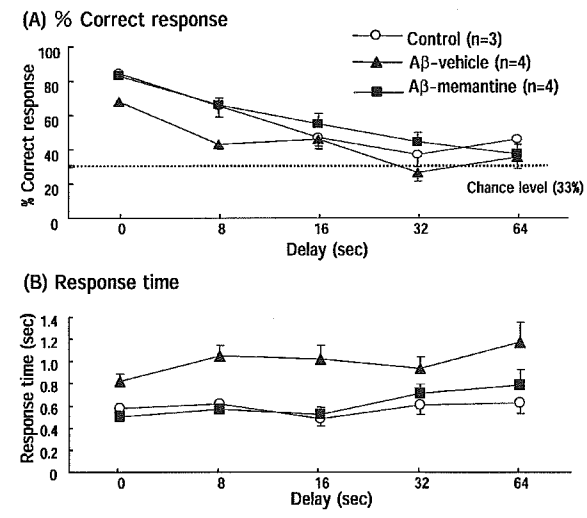

Fig. 4. Effect of memantine on Aβ-induced impairment of DMTP performance from days 51 to 72 following bilateral microinjections of Aβ1-40 into the hippocampus. (A) Percent correct response and (B) response time. Memantine was continuously infused at a dose of 20 mg/(kg day) with the Alzet osmotic pump for 4 weeks from 3 days before to 25 days after Aβ1-40 infusion. Values indicate the mean ± S.E. ($n=3$ for control group, $n=4$ for Aβ-vehicle and Aβ-memantine groups). A two-way ANOVA revealed a significant effect of group on the percent correct response [$F(2,41)=9.448$, $P<0.001$] and response time [$F(4,41)=9.012$, $P<0.001$].

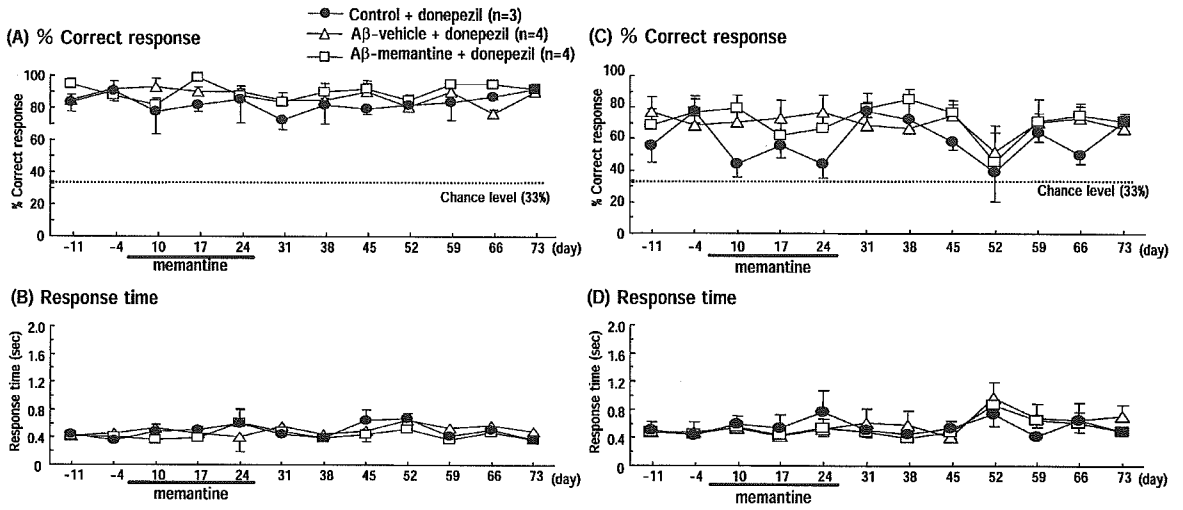

Fig. 5. Effect of donepezil on DMTP performance at the 0-s (A and B) and 8-s delay (C and D) following bilateral microinjections of Aβ1-40 into the hippocampus in control, Aβ-vehicle and Aβ-memantine groups. (A and C) Percent correct response and (B and D) response time. Donepezil (2.5 mg/kg, p.o.) was administered to all animals in the control, Aβ-vehicle and Aβ-memantine groups 1 h before the test sessions. Values indicate the mean ± S.E. ($n=3$ for control group, $n=4$ for Aβ-vehicle and Aβ-memantine groups).

the test session. It appears that donepezil ameliorated the Aβ-induced impairment of DMTP performance on days 51–72. A one-way ANOVA with repeated measures revealed that there were no significant effects of group on DMTP performance from days 52 to 73 at the 0-s (Fig. 5A for choice accuracy: $F(2,8)=2.996$, $P>0.05$; Fig. 5B for response time: $F(2,8)=3.427$, $P>0.05$) and 8-s delay (Fig. 5C for choice accuracy: $F(2,8)=1.304$, $P>0.05$; Fig. 5D for response time: $F(2,8)=0.218$, $P>0.05$). The effect of donepezil on the Aβ-induced impairment of DMTP performance was analyzed by comparing the average DMTP performance in the Aβ-vehicle group from days 51 to 72 when distilled water but not donepezil was administered 1 h before test sessions (Fig. 6). A two-way ANOVA revealed significant effects of delay (Fig. 6A for choice accuracy: $F(4,164)=36.860$, $P<0.0001$; Fig. 6B for response time: $F(4,163)=4.170$, $P<0.01$) and group (Fig. 6A for choice accuracy: $F(2,41)=7.598$, $P<0.01$; Fig. 6B for response time: $F(2,41)=6.547$, $P<0.01$), but not delay × group interaction (Fig. 6A for choice accuracy: $F(8,164)=1.630$, $P>0.05$; Fig. 6B for response time: $F(8,164)=1.201$, $P>0.05$). A post hoc analysis with the Student–Newman–Keuls test revealed that the Aβ-vehicle group showed a significant decrease in choice accuracy ($P<0.05$) and an increase in response time ($P<0.05$) compared with the control group. Donepezil significantly ameliorated the Aβ-induced decrease in choice accuracy ($P<0.05$) and increase in response time ($P<0.05$).

Fig. 7 shows the effects of memantine, donepezil and their combination on the Aβ-induced impairment of DMTP performance at the 0- or 8-s delay from days 51 to 73 following bilateral microinjections of Aβ1-40 into the hippocampus. Bilateral microinjections of Aβ1-40 into the hippocampus in rats caused a significant decrease in choice accuracy (Fig. 7A, $F(5,82)=11.355$, $P<0.05$ by post hoc comparison) and a significant increase in response time (Fig. 7B, $F(5,82)=7.915$, $P<0.05$ by post hoc comparison) in the DMTP task at the 0-s delay. The Aβ-induced impairment of choice accuracy and response time was ameliorated

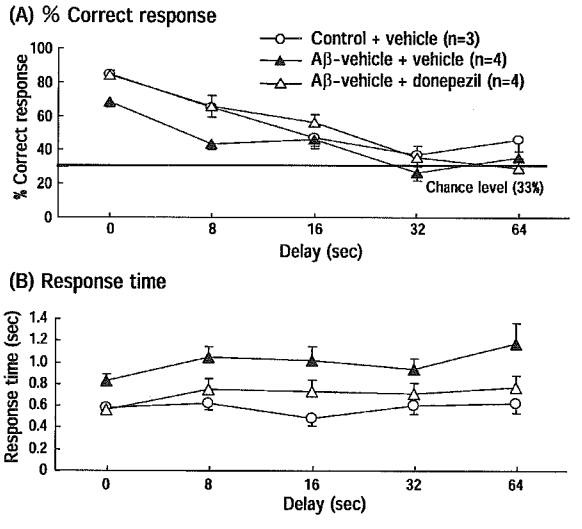

Fig. 6. Effect of donepezil on Aβ-induced impairment of DMTP performance from days 51 to 73 following bilateral microinjections of Aβ1-40 into the hippocampus. (A) Percent correct response and (B) response time. Donepezil (2.5 mg/kg) or the vehicle was administered p.o. 1 h before the DMTP test sessions. Values indicate the mean ± S.E. ($n=3$ for control group, $n=4$ for Aβ-vehicle and Aβ-donepezil groups). A two-way ANOVA revealed a significant effect of group on percent correct response [$F(2,41)=7.598$, $P<0.01$] and response time [$F(2,41)=6.547$, $P<0.01$].

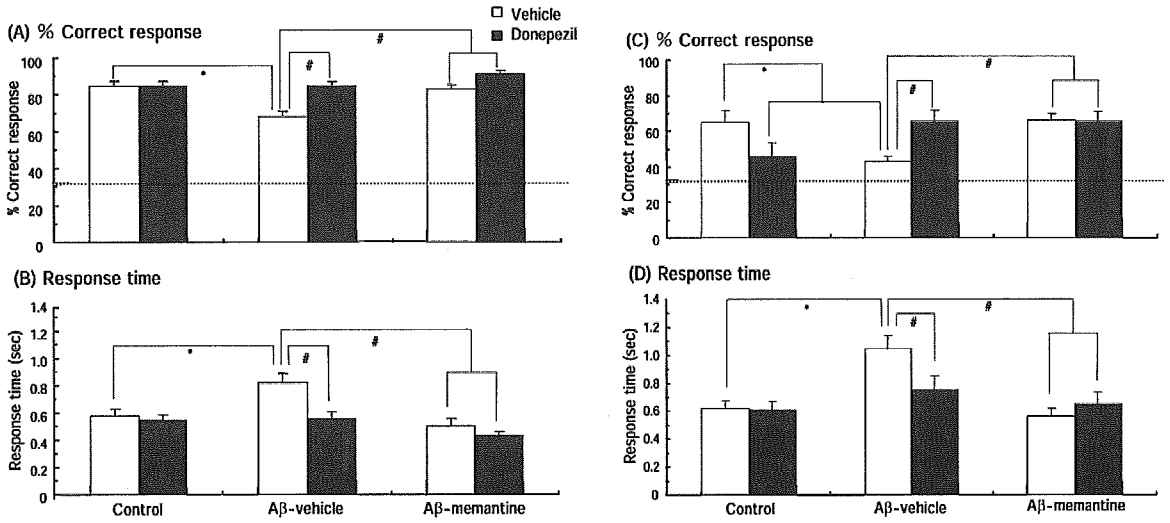

Fig. 7. Effects of memantine, donepezil and their combination on Aβ-induced impairment of DMTP performance from days 51 to 73 following bilateral microinjections of Aβ1-40 into the hippocampus at the 0-s (A and B) and 8-s delay (C and D). (A and C) Percent correct response and (B and D) response time. Memantine (20 mg/(kg day)) was continuously infused s.c. with the Alzet osmotic pump for 4 weeks from 3 days before to 25 days after Aβ1-40 infusion. Donepezil (2.5 mg/kg) or distilled water was administered 1 h before the test session. Values indicate the mean ± S.E. ($n=3$ for control group, $n=4$ for Aβ-vehicle and Aβ-memantine groups). $^{*}P<0.05$ vs. control-distilled water group. $^{\#}P<0.05$ vs. Aβ-distilled water group.

by memantine ($P<0.05$) and donepezil ($P<0.05$). Almost the same results were observed at the 8-s delay (Fig. 7C for choice accuracy: $F(5,82)=4.155$, $P<0.01$; Fig. 7D for response time: $F(5,82)=5.007$, $P<0.001$). However, donepezil significantly decreased choice accuracy ($P<0.05$ by post hoc comparison) without affecting the response time at the 0-s delay in control animals although the same treatment in Aβ-injected rats resulted in an improvement of DMTP performance. The combination of memantine and donepezil failed to produce any additive or synergic effects.

On days 79 and 80 (7 days after the last test session of the DMTP task) after the bilateral microinjections of Aβ1-40 into the hippocampus, the locomotor activity of the animals was measured. Locomotor activity in the control, Aβ-vehicle and Aβ-memantine groups was 687 ± 33, 616 ± 58 and 732 ± 38 counts/(60 min), respectively. There was no difference in locomotor activity among the three groups of animals ($F(2,19)=1.394$, $P>0.05$).

Histological examination by Nissl staining indicated Aβ-induced neurodegeneration in the CA1 and CA3 subfields of the hippocampus. Moreover, it appeared that memantine treatment provided neuroprotection against Aβ-induced neurodegeneration in the hippocampus (Fig. 8).

4. Discussion

In the present study, we have demonstrated that memantine prevented the development of, while donepezil symptomatically alleviated, Aβ1-40-induced short-term memory deficits in rats that received bilateral microinjections of aggregated Aβ1-40 into the CA1 and CA3 subfields of the hippocampus. Although we did not examine the mechanisms underlying Aβ-induced short-term memory deficits, it has been shown that water-reconstituted Aβ1-40, but not water alone or Aβ1-28, injected into the hippocampus is associated with marked neurodegeneration that exhibits the characteristics of apoptosis [20]. Furthermore, it has been reported that memantine protects against neuronal degeneration induced by Aβ1-40 [19]. Accordingly, it is plausible that prevention by memantine of Aβ-induced short-term memory impairment may be associated with its protection against Aβ-induced neurodegeneration in the hippocampus.

Miguel-Hidalgo et al. [19] have reported that neither the acquisition nor retention of the spatial discriminative learning in a T-maze is impaired in Aβ1-40-treated animals despite the neurodegeneration of the CA1 subfield of the hippocampus. The discrepancy may be due to the differences in the amount of Aβ1-40 injected into the hippocampus, injection site or especially the timing and difficulty of the behavioral task. While Miguel-Hidalgo et al. [19] examined learning and memory on day 8 after the Aβ injection when we failed to find any impairments of DMTP performance, an impairment of short-term memory was evident in Aβ1-40-treated animals more than 50 days after Aβ injections.

Consistent with the present findings, previous studies have demonstrated that injection of aggregated Aβ into the hippocampus results in a delayed (approximately 30 days after the treatment) memory impairment [2,24,31], whereas Aβ injection into the nucleus basalis impaired cognitive function at 60 days post-injection [8]. Richardson et al. [31] have suggested that delayed behavioral effects are due to damage to neurons following Aβ-induced activation of glial cells. They showed that aggregated Aβ was present in the brain

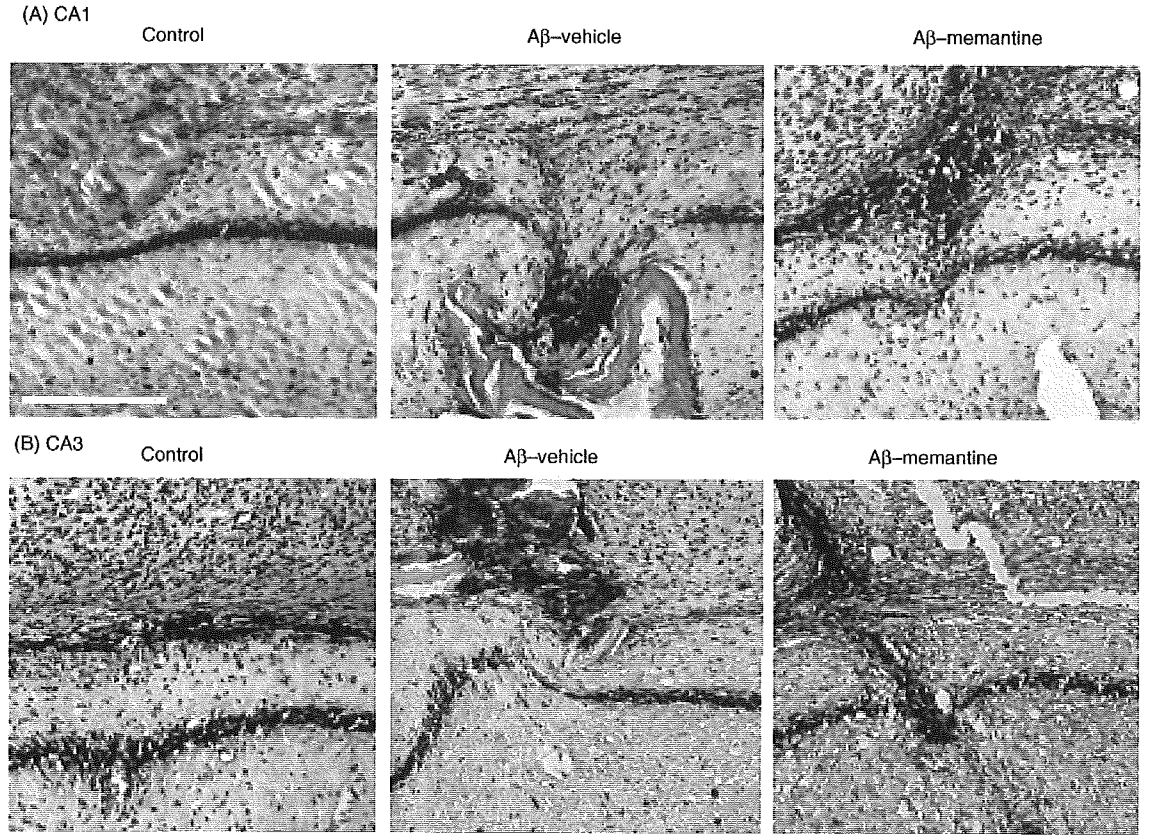

Fig. 8. Micrographs of Nissl-stained sections on day 82 following bilateral microinjections of aggregated Aβ1-40 into the CA1 and CA3 subfields of the hippocampus. (A) CA1 and (B) CA3. Scale bar, 200 μm.

of Aβ-injected rats 80 days post-injection, and substantial astrogliosis was evident in rats with aggregated Aβ. In this regard, memantine has been reported to suppress the gliosis in the brain of rats treated with ibotenic acid [1] or Aβ1-40 [19].

Under the experimental conditions in the present study, acute oral administration of donepezil almost completely ameliorated the short-term memory deficit on the test day in Aβ-injected rats, but no ameliorating effects were evident in the next DMTP test session when the animals were administered distilled water but not donepezil, indicating that donepezil produced a symptomatic alleviation of Aβ-induced short-term memory impairment. Furthermore, it is suggested that cholinergic activation by cholinesterase inhibitors can restore the Aβ-induced memory dysfunction. This assumption may be consistent with the fact that acute or chronic Aβ infusion into the brain results in an impairment of the cholinergic neuronal system [8,12,36]. However, it has been reported that donepezil shows high affinity for sigma receptors [13]. Furthermore, an interaction of donepezil with NMDA receptors could be supposed due to the similarities of the chemical structure with ifenprodil. Thus, further investigation with other selective cholinesterase inhibitors such as rivastigmine [7] is necessary to conclude the effectiveness of cholinesterase inhibitors on Aβ-induced memory dysfunction.

A recent study has indicated that donepezil protects cortical neurons against glutamate neurotoxicity via α4β2- and α7-nicotinic acetylcholine receptors [34]. Accordingly, we cannot exclude the possibility that donepezil may have neuroprotective effects on Aβ-induced memory impairment under different experimental conditions. Continuous infusion of denepezil could lead to neuroprotective effects through the activation of nicotinic and/or muscarinic acetylcholine receptors.

The combination of memantine and donepezil failed to produce any additive or synergic effects on Aβ-induced short-term memory impairment although a clinical study has demonstrated that memantine treatment resulted in significantly better outcomes than placebo in patients with moderate to severe AD already receiving donepezil [35]. This is probably due to the ceiling effect because each drug showed almost complete amelioration of memory deficits in Aβ-treated animals. Thus, further studies are required to investigate the effect of co-treatment of memantine with donepezil on Aβ-induced memory impairment.

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生化学からみたニコチン性アセチルコリン受容体とその機能

Neurochemical function of nicotinic receptors in central nerves system

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Summary

アセチルコリン(ACh)は脳内の神経伝達物質の1つであり，アルツハイマー病(AD)をはじめとする認知症の病態生理に重要である。AChの受容体は，ムスカリン性ACh受容体とニコチン性ACh受容体(ニコチン受容体)の2つに分けられる。ADではいずれの受容体も減少していることが知られているが，特にニコチン受容体の減少がADの病態に深く関わっていることが示唆されている。ニコチン受容体の賦活化は，種々の神経伝達系を増強し，記憶力の改善や注意力の向上を引き起こす。それゆえ，ADの薬物療法の1つに，ニコチン受容体が創薬ターゲットに挙げられ，最近では，アロステリック部位を介したニコチン受容体の機能が注目されている。一方，分子生物学の進歩に伴い，ニコチン受容体に特異的な遺伝子改変マウスが作製された。これらの遺伝子改変動物を用いた研究によって，ニコチン受容体の発現部位やその機能が解明されつつある。

Key words

●ニコチン性アセチルコリン受容体
●神経伝達物質
●アロステリック部位

I はじめに

近年のニコチン性アセチルコリン受容体(ニコチン受容体)に関する研究は，サブタイプの分類，遺伝子発現機構の解明などをはじめとして急速な進歩を遂げており，ニコチン受容体の薬理学的役割に関して単なる神経伝達物質受容体としての概念を超える多様な機能が提唱されるようになってきた。ニコチン受容体は，運動神経終板，自律神経節ばかりでなく中枢神経系や免疫系においても存在しており，ニコチン受容体の多様な生理機能の制御は，発現部位や種々のニコチン受容体のサブタイプの違いによって異なっていることが指摘されている。臨床においては，アルツハイマー病(Alzheimer's disease；AD)やパーキンソン病(Parkinson's disease；PD)などの神経変性疾患や免疫疾患など種々の疾患においてニコチン受容体が新たな創薬ターゲットとして注目されるようになっている。ADの中核症状である記銘力障害や認知機能障害が認められる患者の脳において，アセチルコリン(ACh)が結合する受容体であるムスカリン受容体とニコチン受容体のうち，ニコチン受容体数が著しく減少している。ADの早い時期にニコチン受容体の減少が始まること，ニコチン受容体数の減少が認知症症状の重症度と相関すること，老人斑や神経原線維変化

の周囲でニコチン受容体数の減少が著しいことが報告されており[1)-3)]，他の神経伝達系よりもニコチン受容体とADとの関連が強く示唆されている。一方，多くの疫学的研究からADやPDなどの神経変性疾患の発症率はタバコの喫煙率と負の相関があり，喫煙者と比較して非喫煙者ではADやPDが約3倍も高いことが報告されている[4)]。ニコチンには，意識レベルや注意力を改善する効果がヒトでも認められ，ニコチンパッチを用いた検討においても認知機能の改善が報告されている[1)5)6)]。これらの研究は，ニコチン受容体が認知機能に直接関与することを示唆している。最近では，ニコチン受容体上にアロステリック部位が存在することが多数報告され[2)3)7)8)]，アロステリック作用を有する新しいリガンドの発見やADの治療薬の開発に向けて注目を浴びている。

本稿では，ニコチン受容体のサブタイプの機能について生化学，生理学，薬理学的観点から，ADにおける中枢神経系のニコチン受容体の役割について概説する。

II ニコチン受容体の特徴

ニコチン受容体は5つのサブユニットからなるイオンチャネル型受容体であり，これまでに，少なくとも9つのαサブユニット(α2～α10)と3つのβサブユニット(β2～β4)とが同定されている[9)]。ほとんどの神経のニコチン受容体はαとβの両方のサブユニットにより構成され，その化学量論において，これらのサブユニットの典型的な比は2：3と考えられている。しかし，α7，α8，およびα9サブユニットのみから構成されるサブタイプもある。最近，それぞれのサブユニットやサブタイプの脳内分布が明らかとなり，脳においてα4β2やα7受容体が比較的高密度・広範囲に発現していることが確認されている。このようなニコチン受容体は，脳内において神経のシナプス前終末やシナプス後膜に存在し，ドパミン，グルタミン酸，γ-アミノ酪酸(GABA)などの神経伝達物質の遊離や細胞内シグナル伝達機構の活性化などに関与している。シナプス後膜である細胞体のニコチン受容体を活性化すると，細胞内のカルシウムレベルが上昇し，カルシウムを介するシグナルが核内へ伝達される(図1)。ニコチン受容体の活性化による細胞内カルシウムレベルの上昇の機構として，電位依存性カルシウムチャネルの活性化による機構，ニコチン受容体を介して直接的に流入する機構，細胞へ流入したカルシウムが引き金になり細胞内カルシウム小胞からのカルシウム放出機構が考えられている。このような細胞内カルシウムレベルの上昇は，PI3K(phosphatidylinositol 3-kinase)/Akt経路の活性化[7)]，カルシニューリンの活性化[10)]，MAPK/ERK(mitogen-activated protein kinase/extracellular signal-regulated kinase)シグナルの活性化[10)]といったシグナル伝達を亢進させる。特に細胞の生存に関わるPI3K/Akt経路は，*in vitro*実験においてニコチン受容体(特にα7受容体)を介した神経保護作用のメカニズムの一端を担っていることが示唆されている[7)]。α4やα7受容体を持続的に活性化すると，NMDA(N-methyl-D-asparate)受容体刺激によるカルシウム流入により誘発される一酸化窒素ラジカルの産生に対して抑制作用を示すことも報告されている[11)]。しかし，このようなニコチン受容体を介した細胞内シグナル伝達機構の研究は，ニコチンやニコチン受容体の作動薬の急性効果について主になされており，脳部位別(細胞別)，種差，研究グループによって結果が異なっている。臨床試験や動物個体を用いた*in vivo*実験において，ニコチンやアセチルコリンエステラーゼ(AChE)阻害薬を慢性的に処置すると，ニコチン受容体の脱感作が生じることが報告されている[8)]が，ニコチン受容体の細胞内シグナル伝達へのこれら薬物の慢性処置による影響については詳細に検討されていない。

III ニコチン受容体を介した神経伝達物質の調節

AChは，代表的な興奮性神経伝達物質の1つである。神経シナプス終末部において，ニコチン受容体を介して細胞内カルシウムレベルが上昇すると，シナプス小胞に貯蔵された神経伝達物質が放出される(図2)。ニコチンはドパミン遊離調節に関与していることから，PDにおいて中脳辺縁系ドパミン作動性神経系へのニコチンの影響について多数の研究が行われている。一方，ニコチン

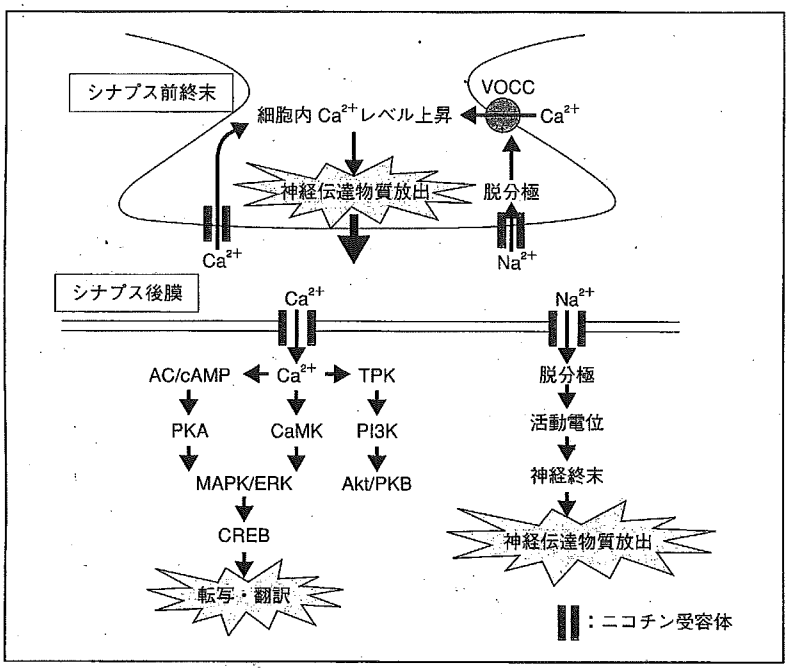

図1　ニコチン受容体の情報伝達機構
AC：adenylate cyclase, Akt/PKB：protein kinase B, CaMK：Ca^{2+}/calmodulin-dependent protein kinase, CREB：cAMP-responsive element-binding protein, MAPK/ERK：mitogen-activated protein kinase/extracellular signal-regulated kinase, PI3K：phosphatidylinositol 3-kinase, PKA：protein kinase A, TPK：tyrosine protein kinase, VOCC：voltage-operated Ca^{2+} channel
（文献23）より一部改変，引用）

自身は，認知機能改善効果のみならず依存性物質として作用することが報告されている。実験動物においてニコチンは依存に関連のある側坐核内のドパミン遊離を投与後2時間にわたり増加させる[12]。われわれの研究室においては，AChE阻害薬を投与すると，認知機能を司っている海馬内のドパミン遊離が増加し，この増加が学習記憶改善効果の一端を担っている可能性を見出している。一方，電気生理学的研究においては，ニコチンを中脳スライスに添加すると，一時的にドパミン作動性神経の発火頻度が増加するが，ニコチン刺激の数分後にはニコチンに対する反応性が減弱する[13]。このようなドパミン作動性神経の反応性の減弱には，$\alpha4\beta2$ニコチン受容体の脱感作が関与していることが報告されている[14]。ニコチン受容体を介した一時的な神経刺激作用はドパミン作動性神経だけでなく，GABA作動性神経においても観察される[15]。また，腹側被蓋野（ventral tegmental area；VTA）に投射しているグルタミン酸作動性神経のシナプス前終末に存在する$\alpha7$受容体を介して，ニコチンはグルタミン酸の遊離を増加させる[16]。このグルタミン酸作動性神経の活性化は，ドパミン作動性神経やGABA作動性神経とは異なり脱感作を受けないことから，ドパミン作動性神経の長期的な活性化は，ドパミン作動性神経に対する直接的な経路とグルタミン酸作動性神経およびGABA作動性神経を介する経路の総和によって生じている可能性が考えられている。以上のように，ニコチン受容体は，発現部位やサブタイプの違いを介してさまざまな神経伝達物質の遊離を調節し，その結果，記憶力の向上，注意力の改善，意識レベルの向上といった作用を示すと考えられる[1)6)]（図2）。

Ⅳ　ニコチン受容体とallosteric potentiating ligand（APL）

上述したように，ニコチン受容体はカチオンチャネルとして細胞内へのナトリウムやカルシウムイオンの流入を調節することで，神経伝達物質の遊離を調節し，脳機

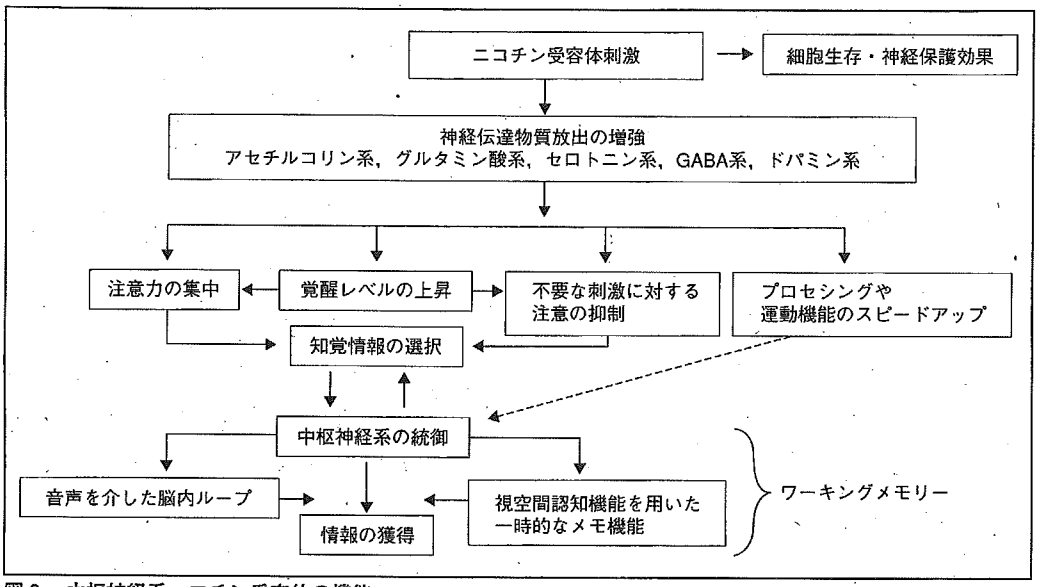

図2　中枢神経系ニコチン受容体の機能

（文献1）および6）より一部改変，引用）

能の維持に重要な役割を担っている。このようなニコチン受容体を介する作用は，AChやニコチンが受容体のニコチン結合部位に作用することにより引き起こされるが，最近，その結合部位以外に，アロステリック結合部位が存在することが多数報告されている[2)3)7)8)]。ニコチン受容体上のアロステリック部位は，αサブユニットの細胞外のN末端ドメイン上に存在すると考えられている[2)17)]。APLの可能性がある代表的なものとして，フィゾスチグミン，リドカイン，ガランタミン，さらに内因性因子としてセロトニンなどが報告されている[2)17)]。PC12細胞において，AChによる電流応答が低濃度のセロトニンを併用すると約60%以上増強し，このセロトニンの増強作用は，αサブユニットに特異的な中和抗体であるFK1抗体を前処置することによって抑制される[2)]。このことは，セロトニンはニコチン受容体のアロステリック部位に結合することによってACh誘発電流応答を増強する可能性を示唆している。しかし，高用量のセロトニンは，ACh誘発電流応答に対して逆に抑制効果を示すことから，APLとしてのセロトニンの作用については詳細な検討が必要である。AChE阻害薬であるガランタミンはAPL作用を有している新しいAD治療薬として注目されている。現在，ADの治療にAChE阻害薬が用いられているが，AChE阻害薬は一般的に長期にわたる治療効果はないといわれている[3)]。動物実験において，ニコチンやニコチン作動薬はAChと同じ結合部位に作用するため，これらを持続的に治療に用いればニコチン受容体の脱感作が生じ，最終的には薬物耐性が発現して薬効が現れにくくなる[8)]。

APL作動薬であるガランタミンは，シナプス前神経終末のニコチン受容体のアロステリック部位に結合し，ACh，グルタミン酸，GABAなどの多様な神経伝達物質の放出を促進する[3)18)]。一方，ガランタミンがシナプス後膜のニコチン受容体のアロステリック部位に作用することで$\alpha4\beta2$や$\alpha7$受容体を活性化させる。アミロイドβ蛋白によるグルタミン酸誘発神経細胞死の増強作用に対して，ガランタミンはそれ単独で保護効果を示し，その作用は，ニコチン受容体の下流シグナルであるPI3K/Aktシグナルの増強を介して発現している可能性が報告

されている[10]。このようにアロステリック部位を刺激することで，ニコチン受容体に効率よくAChが結合し，ナトリウムやカルシウムイオンの流入が円滑となり神経細胞間の電気的シグナルが促進される。興味深いことに，AD患者に対してガランタミンを36ヵ月間長期投与した臨床試験において，18ヵ月もの間，認知機能の低下が改善されることが報告されている[19]。長期にわたり効果が持続したことは，ニコチン受容体の脱感作が認められなかったことを意味しており，allosteric modulatorとしての作用であるニコチン受容体の賦活化や神経伝達物質の遊離の調節およびAChE阻害薬としての作用が総合的に影響したものと考えられる。以上のように，アロステリック部位を介してニコチン受容体の機能を調節することで，ADをはじめとした種々の疾患の治療ができる可能性を秘めており，新たなるAPL作動薬の開発やセロトニンに追随する内因性因子の発見が期待される。

Ⅴ 遺伝子改変マウスにより明らかになったニコチン受容体サブタイプの生体内での異なった役割

近年，分子生物学的技術の著しい発展により，ニコチン受容体サブユニットの変異マウスが開発され，多くの研究者によって種々のニコチン受容体サブタイプの生体内における機能について研究されている[9][20]（表1）。α7サブユニット欠損マウスの脳において，α-bungarotoxin（α7サブユニット構成ニコチン受容体阻害剤）結合活性が著しく低下しているが，ニコチン結合活性はあまり変化していない。一方，ニコチン結合活性はα4サブユニットの欠損では著明に低下し，β2サブユニットの欠損ではほとんど消失する。このことから，脳のニコチン誘発反応のほとんどはα4β2受容体由来と考えられる。β2あるいはβ4サブユニットの欠損では正常に出生・発育するが，これら遺伝子を両方欠損させると出生後しばらくして死亡する。α3の単独欠損でも同様に生後死に至る。これらのことから，β2とβ4サブユニットは代償機能をもち，自律神経系においてα3サブユニットと共役していることが示唆されている[9]。一方，α4あるいはα7サブユニットを欠損しても普通に出生・発育する[9]。

脳においてα4β2やα7受容体が比較的高密度・広範囲に発現していることから，これらサブユニットを欠損させたマウスを用いた研究を取り上げ，その機能について以下に紹介する。

表1　ニコチン受容体サブユニット遺伝子改変マウスに認められるニコチンの効果

サブユニットの欠損	基本的な機能変化	ニコチンによる行動学的変化	生存
α3	自律神経系機能不全，巨大膀胱症，瞳孔散大，上頸神経節のアセチルコリン反応の変化	ND	生後1～7日
α4	高親和性ニコチン結合能の低下	ニコチンの抗侵害効果の減弱	正常
α7	海馬神経のニコチン電流応答の脱感作の欠損	行動に変化なし	正常
α7 Leu247Thr	海馬神経のニコチン電流応答の脱感作の遅延	ND	生後24時間
α9	蝸牛の内有毛細胞の分布の異常	蝸牛反応の抑制	正常
β2	高親和性ニコチン結合能の低下，ニコチン誘起ドパミン遊離の低下，老化現象	Passive avoidance潜在時間の増加 ニコチン自己投与の低下 ニコチンの抗侵害効果の減弱	正常
β3	線条体シナプトソーム中のα-conotoxin MII感受性受容体の低下	自発運動量の増加	正常
β4	上頸神経節におけるニコチン電流応答の減弱	ND	正常
β2/β4	自律神経系機能不全	ND	生後1～3週

ND：not determined

（文献9）より一部改変，引用）

1．α4およびβ2サブユニット欠損マウス

ニコチンは新規環境下における探索運動を抑制するが，習慣性環境下ではドパミン作動性神経系の活性化により運動量を増加させる。β2サブユニット欠損マウスは，野生型マウスと比較して習慣性環境下における自発運動量が減弱しており，α4サブユニット欠損マウスはニコチンの自発運動抑制効果が減弱していることが報告されている[20][21]。これらの結果は，α4およびβ2サブユニットを含むニコチン受容体は自発運動において重要な役割を果たしていることを示唆している。この要因の1つとして，野生型マウスをニコチン刺激するとシナプトソームからドパミンやGABAが遊離されるのに対し，β2サブユニット欠損マウスではそのようなニコチンの作用は認められないことから，β2サブユニットがニコチン刺激による神経伝達物質の遊離に関与していることが示唆される。興味深いことに，β2サブユニット欠損マウスは，老齢に伴い海馬CA3領域の神経細胞死や皮質の萎縮といった神経変性が野生型マウスと比較して増加していることが確認されている[20]。それゆえ，水迷路試験や恐怖条件付け試験などの学習記憶試験において，β2サブユニット欠損マウスは野生型マウスと比較して明らかな学習記憶障害を示す。α4サブユニット欠損マウスは，メタンフェタミン誘発神経変性に対するニコチンの保護効果が消失していることが報告されている[20]。これらの結果は，α4およびβ2サブユニット欠損マウスが，ADやPDを代表としたドパミン作動性神経系の神経変性疾患モデルとして有用である可能性を示している。

2．α7サブユニット欠損マウス

中枢神経系において胎児13日目よりα7サブユニットmRNAの発現が確認されていることから，α7受容体は脳の発達段階に関与している可能性が示唆されている。α-bungarotoxin感受性チャネルによって神経の成長が調節されていること，α7受容体を介したカルシウムイオン流入は細胞機能の調節に影響を及ぼすことが報告されている[9]。しかし，α7サブユニット欠損マウスは野生型マウスと比較して，脳の細胞数や層構造に大きな違いが認められず，皮質一次体性感覚野のバレル構造も存在する。海馬における神経細胞の局在や海馬内神経ネットワークに異常がないことも報告されている[9]。それゆえ，胚形成時期におけるα7サブユニットは細胞移動や脳の発育にとってさほど影響を示さない可能性が考えられている。α7サブユニットを部分的に改変することで，作用薬の親和性の増強や脱感作の減少が認められる遺伝子改変マウス(α7 Leu247Thr)では，皮質一次体性感覚野の広範囲にわたってアポトーシスが誘導され，生後24時間以内に死に至ると報告されている[9]。α7サブユニットに脱感作が急速に起こるのは，ACh受容体刺激による莫大なカルシウムイオン流入を調節する役割を担っており，脱感作はその後生じる莫大なカルシウムイオン流入に対する防御的連絡応答として働き，莫大なカルシウムイオン流入による神経細胞死に対して保護的に働いていると考えられている[9][22]。

Ⅵ おわりに

近年，分子生物学的手法の発展により，脳内におけるニコチン受容体に関する研究が進展しており，受容体の個々のサブタイプの局在や機能の違いなどが解明されつつある。ニコチン受容体は学習・記憶といった脳機能に最も深く関与する受容体といっても過言ではなく，ADをはじめ，統合失調症，PD，注意欠損多動障害に認められる学習障害に共通したターゲット受容体である。今後，ニコチン受容体のサブタイプの研究やアロステリック部位を介したニコチン受容体の機能解析が進み，こうした研究が種々の疾患治療に大いに役立つことを期待する。

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アルツハイマー病の病態とその治療戦略

鍋島俊隆*

Ⅰ．アルツハイマー病の現状と病態

図1は65歳以上の認知症（痴呆）老人の将来推計です。現在，日本の社会は166万人の認知症患者を抱え，女性と男性の比は約2対1になっています。これが2036年になると355万人になります。この対策が緊急の課題になっております。

Gersingらの報告によると，認知症患者の中でアルツハイマー病の患者は55％と半数以上を占めます。脳血管性認知症はリスクファクターがよくわかってきてコントロール可能になりましたが，アルツハイマー病はまだ対策が十分できておらず，重点的に研究を行う必要があると思われます。

アルツハイマー病は記憶障害が中核にありますから，記銘力，空間的・時間的見当識の障害が出ます。そして進行性の神経変性疾患です。ですから，この進行をどうとめるかが治療戦略の1つになります。病理学的，神経学的変化としては，アミロイドβ（Aβ）蛋白の異常な産生と沈着があります。Aβ蛋白が変性性・進行性の認知症に関与しているということで，将来の戦略はAβ蛋白をターゲットとするのが，原因療法につながると考えられます。それから，Aβ蛋白はタウ蛋白のリン酸化を異常に引き起こすこともわかっており，その結果，神経原線維変化が起こり，神経がよじれて死んでいきます。ですから，ターゲットをAβ蛋白に絞ることが非常に大事なのです。

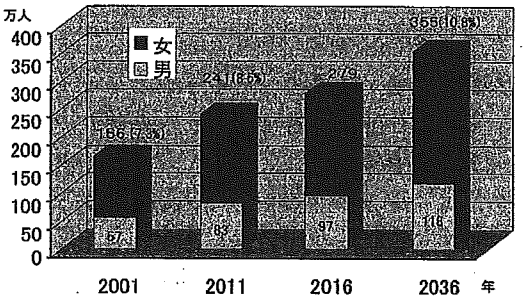

図1　65歳以上の認知症（痴呆）性老人の将来推計
％：65歳以上の人口に占める割合
東京武蔵野病院　大塚俊男（2001）

アルツハイマー病では脳神経細胞が萎縮・脱落し，脳の血流量やブドウ糖利用率も低下します。脳血流は酸素とブドウ糖を脳に運んでいます。脳のエネルギーは酸素とブドウ糖からできるATPに依存しているので，脳機能が落ちてきます。

それから，神経伝達関係では，アルツハイマー病の初期からコリン作動性神経系の障害が認められています。現在の治療はここをターゲットにしているわけですが，病気が進んでくると神経細胞がどんどん死んでいく病気ですから，モノアミン作動性神経系，GABA作動性神経系，グルタミン酸作動性神経系に影響が出てきます。ですから，これらをターゲットとする薬も最近は開発されて

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