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Effect of nicotine on neuronal dysfunction induced by intracerebroventricular infusion of amyloid-β peptide in rats

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Abstract. – OBJECTIVE: The aim of the study was to investigate the effects of nicotine on learning and memory deficits induced by intracerebroventricular infusion of amyloid-β peptide (Aβ) in rats.

MATERIALS AND METHODS: Neuronal dysfunction in rats was induced by an infusion of $A\beta_{1-42}$ (20 μg/body, over 3 days) into right ventricle. Nicotine was administered intraperitoneally to the rats at 0.2 mg/kg, once a day for 9 weeks beginning 3 weeks after the Aβ infusion. Learning and memory functions were examined by behavioral tests including Morris water maze task performed on days 87-90. As biochemical analyses, choline acetyltransferase (ChAT) activity and hemicholinium-3 (HC-3) binding were measured in brain tissues after the behavioral examination.

RESULTS: The Aβ infusion induced significant learning and memory deficits in rats, judging from the behavioral tests. Treatment of the rats with nicotine significantly improved the Aβ-induced learning and memory deficits in water maze task. The Aβ infusion also decreased significantly not only the level of ChAT activity in posterior cortex and striatum, but the HC-3 binding in anterior cortex, posterior cortex, and hippocampus. The nicotine treatment did not reverse the level of ChAT but significantly inhibited the decrease in HC-3 binding, indicating improvement of cholinergic function without affecting the number of ACh terminals.

CONCLUSIONS: Nicotine ameliorated learning and memory deficits in the $A\beta_{1-42}$-induced animal model, which is mediated, at least in part, by enhancement of cholinergic neurotransmission. nAChR ligands including nicotine is thought to be useful as a treatment for Alzheimer's disease.

Key Words:
Nicotine, Amyloid-β, Water maze task, Passive avoidance task, Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive decline and ultimately loss of cognitive function[1,2]. The typical features of the brains of AD are the presence of neurofibrillary tangles and deposits of amyloid-β peptide (Aβ) in neocortical brain structure[3]. Aβ is believed to be a pivotal mediator of neuronal degeneration and, thereby, impair cognitive function[4,5] because of its neurotoxic activity. A wide array of antiamyloid and neuroprotective therapeutic approaches is currently under investigation[6].

Nicotinic acetylcholine receptors (nAChRs) are transmembrane oligomeric ligand-gated ion channels of about 300 kDa and expressed widely throughout the central and peripheral nervous systems. To date, seventeen nAChR subunits (α1-α10, β1-β4, γ, δ and ε) have been identified in vertebrate species, all of which, except α8, are expressed in humans and in different mammalian species. nAChRs are divided in two groups, i.e. homopentamers which are formed by five identical subunits, and heteropentamers which are composed by combination of different two subunits[7-9]. It has been known for some time that nAChR density[10,11] as well as acetylcholine (ACh), a ligand for nAChRs[12,13], decreases with disease progression of AD. In addition, Aβ is known to bind and modulate nAChRs[14]. These findings have led to the thought that cholinergic molecular components are pharmaceutical targets for AD. In fact, the current primary treatment for AD is ACh esterase inhibitors that offset the declining cholinergic transmission associated with the disease.

Nicotine, an agonist for nAChRs and the major component of cigarette smoke, improves behavioral impairment and increases nAChRs in transgenic mice that express neuron-specific enolase (NSE)-controlled APPsw[15]. Interestingly, nicotine shows protective activities against neuronal cell damage induced by various stimuli[16,17]. In addition, nicotine has been shown to improve

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cognitive function in the AD patients[18], while in another clinical study, nicotine failed to show a significant effect in enhancing memory[19].

The present study was undertaken so as to assess whether nicotine shows some beneficial effect in an AD-type amnesia model in which not only progressive dysfunction of cholinergic neurotransmission but learning impairments are induced by intracerebroventricular infusion of Aβ peptide[20], and our data showed that nicotine ameliorates the Aβ-induced learning and memory deficits. Our findings suggest the usefulness of nAChR agonists such as nicotine in AD treatment.

Materials and Methods

Animals

Male F344/DuCrj rats, aged 18-weeks-old and weighing 320-360 g at the beginning of the experiments, were obtained from Charles River Laboratories Japan, Inc. (Tokyo, Japan). Experiments were carried out following the NIH guideline for the care and use of laboratory animals, and approved by the Local Ethics Committees of Asubio Pharma Co. Ltd (Kobe, Japan). The rats were housed in an artificially controlled room (23 ± 2°C and 55 ± 15% humidity) with food and water available *ad libitum*, under a 12-h light/dark cycle (lights on at 7.00 a.m.). Experiments were carried out between 8.30 a.m. and 4.30 p.m.

$A\beta_{1-42}$ Peptide-Induced AD model in Rats

The $A\beta_{1-42}$ peptide (ANASPEC Inc., San Jose, CA, USA) were dissolved in sterile bidistilled water, at a concentration of 1 mg/ml. The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Continuous infusion of $A\beta_{1-42}$ (20 μg/3 days/body) was maintained for 3 days by attachment of an infusion kit to an osmotic mini-pump (Alzet 1003D; Alza, Cupertino, CA, USA). The infusion kit was implanted into the right ventricle (1.2 mm posterior to the bregma, 1.5 mm lateral to the midline, 4.0 mm ventral to the surface of the skull, according to the brain atlas of Paxinos and Watson). The species of $A\beta_{1-42}$ infused was considered to be mainly a mixture of soluble monomers and oligomers, since the infusion was started in a few hours after the preparation without incubation of the solution. In the sham operation group, the infusion kit was just implanted. Nicotine (0.2 mg/kg, i.p.) or saline was administered once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1-42}$ infusion until the last day of behavioral assessment. The dose of nicotine was determined based on the result of previous pharmacological study in rats conducted in our company[21]. The same rat set was used for passive avoidance task, water maze task, and choline acetyltransferase (ChAT) activity/hemicholinium-3 (HC-3) binding measurements.

Passive Avoidance Task

Passive avoidance was examined 80-84 days after the start of $A\beta_{1-42}$ infusion using the step-through type apparatus. The apparatus consisted of illuminated (20×10 cm base, 15 cm high) and darkened compartments (25×25 cm base, 25 cm high) with a grid floor and a partition board separating the compartments. In the acquisition trial, each rat was placed in the light compartment and then the board to the dark compartment was opened. When the rat entered the dark compartment, the board was closed and an electroshock (0.6 mA, 3 sec, Shockgenerator-Scrambler, Biomedica Ltd., Osaka, Japan) was delivered through the grid. This training continued until the rat stayed in the light compartment for 150 sec, and the numbers of electroshock treatment was recorded as a parameter for learning ability. The retention trial was carried out 96 hours after the training trial, and the time before the rat entered the dark compartment (step-through latency) was recorded for up to 300 sec as a parameter for memory.

Water Maze Task

Water maze tasks were preformed basically according to the method of Morris[22] using a circular water tank 132 cm in diameter and 60 cm deep. The interior surface of the tank was covered with white tape, and the tank was filled to a depth of 45 cm with water (24 ± 2°C) that was clouded with 0.8 kg of powdered milk. A translucent acrylic platform (10 cm in diameter) was submerged 2 cm below the water surface in the center of one of the four quadrants of the maze. The tank was located in a large room with many extra-maze cues (computer, breeding cages, a calendar, black and white graphics, etc.). The positions of the cues and platform were constant throughout the study. The movements of the animal in the tank were monitored with a video tracking system (VIOS-88, Bio-medica Ltd.) and analyzed with a computer (PC-9801, NEC, Tokyo, Japan).

Water maze performance was assessed from 87 days after the start of infusion of $A\beta_{1-42}$. On the 87th day, as the first acquisition study, each rat was placed into the tank without an escape platform and allowed to swim for 60 sec, and then maze training was begun. In each trial, the rat was put into the water from one of four starting points (north, east, south, and west) on the edge of the tank. The starting point was changed among trials in a quasi-random manner but the escape platform was fixed throughout the experiment. The time required to escape onto the hidden platform was recorded in each trial. If the rat found the platform, it was allowed to remain there for 10 sec. If it did not find the platform within 120 sec, the trial was terminated and the experimenter placed the rat onto the platform and allowed it to stay there for 10 sec. The rats were trained for 4 consecutive days with four trials at 60-sec intertrial intervals each day. Two hours after the final training session, a single probe trial was conducted for accessing memory. The escape platform was removed and each rat was allowed to swim for 60 sec in the maze. The number of crossing the annulus where the platform had been located was recorded.

Preparation of Brain Tissues

After water maze tasks were completed on the day 90, each rat was decapitated under ether anesthesia and the brain was rapidly excised. Anterior cortex, posterior cortex, hippocampus, and striatum were dissected out on ice, and stored at -80°C until the ChAT and HC-3 binding assays.

ChAT Activity

ChAT activity was assayed by the method of Fonnum et al[23]. In brief, tissue samples were homogenized in 20 vol. of ice-cold 10 mM EDTA (pH 7.4) containing 0.5% Triton X-100. The reaction mixture contained 10 μM [^{14}C]-acetyl coenzyme A (acetyl CoA; 148.0 MBq/mmol, NEN, Boston, MA, USA), 25 mM sodium phosphate buffer (pH 7.4), 600 mM sodium chloride, 40 mM EDTA, 100 μM physostigmine, 8 mM choline bromide, 200 μM acetyl CoA, and the tissue homogenate (about 70 μg protein) in a total volume of 100 μl. The sample was incubated for 30 min at 37°C and, and [^{14}C]-ACh was extracted directly into 10 ml of scintillation mixture plus 2 ml of acetonitrile containing 10 mg of tetraphenylboron. The radioactivity was measured with a liquid scintillation counter. Results were expressed as nmol/mg protein/h and indicated as ratios.

HC-3 Binding

HC-3 binding was assayed by the method of Manaker et al[24]. Briefly, tissue samples were homogenized in 20 vol. of ice-cold 10 mM sodium-potassium phosphate buffer (pH 7.4). The tissue homogenate (200-300 μg) was incubated in duplicate in a total volume of 200 μl (150 mM NaCl, 2 nM [^{3}H]-HC-3, ±10 μM unlabeled HC-3) at 25°C for 30 min. After the incubation, the samples were immediately filtered on glass fiber filters (GF/B) which had been presoaked in 0.1% (v/v) polyethylenimine. The filters were rapidly washed 3 times each with 5 ml of ice-cold assay buffer. The radioactivity was measured with a liquid scintillation counter. Results were expressed as fmol/mg protein and indicated as ratios.

Statistical Analysis

Data were presented as mean ± SEM. Statistical analysis was performed by two-way repeated measures ANOVA for acquisition trials in water maze tasks or one-way ANOVA for the other measures, followed by Student's *t*-test (EXSUS version 7.5.2, Arm Systex Co., Ltd, Osaka, Japan); *p* value less than 0.05 was taken as indicative of statistical significance.

Results

Effect of Nicotine on Learning and Memory Deficit in Morris Water Maze Performance in Rat Cognitive Dysfunction Model Induced By Intracerebroventricular Infusion of $A\beta_{1-42}$

Figure 1 shows the effects of nicotine on learning and memory deficits in water maze performance with a rat cognitive dysfunction model induced by intracerebroventricular infusion of $A\beta_{1-42}$. The water maze task was performed for 4 days (87-90 days after the start of $A\beta_{1-42}$ infusion). As shown in Figure 1A, the mean escape latency (MEL) in the water maze task was significantly prolonged by the infusion of $A\beta_{1-42}$, indicating learning impairment. Administration of nicotine (0.2 mg/kg) once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1-42}$ infusion significantly inhibited the $A\beta_{1-42}$-induced prolongation of MEL on days 3 and 4 (Figure 1A). In a single probe trial conducted 2 h after the last acquisition trial on day 4, the number of annulus crossing was markedly decreased by intracerebroventricular infusion of $A\beta_{1-42}$ (Figure

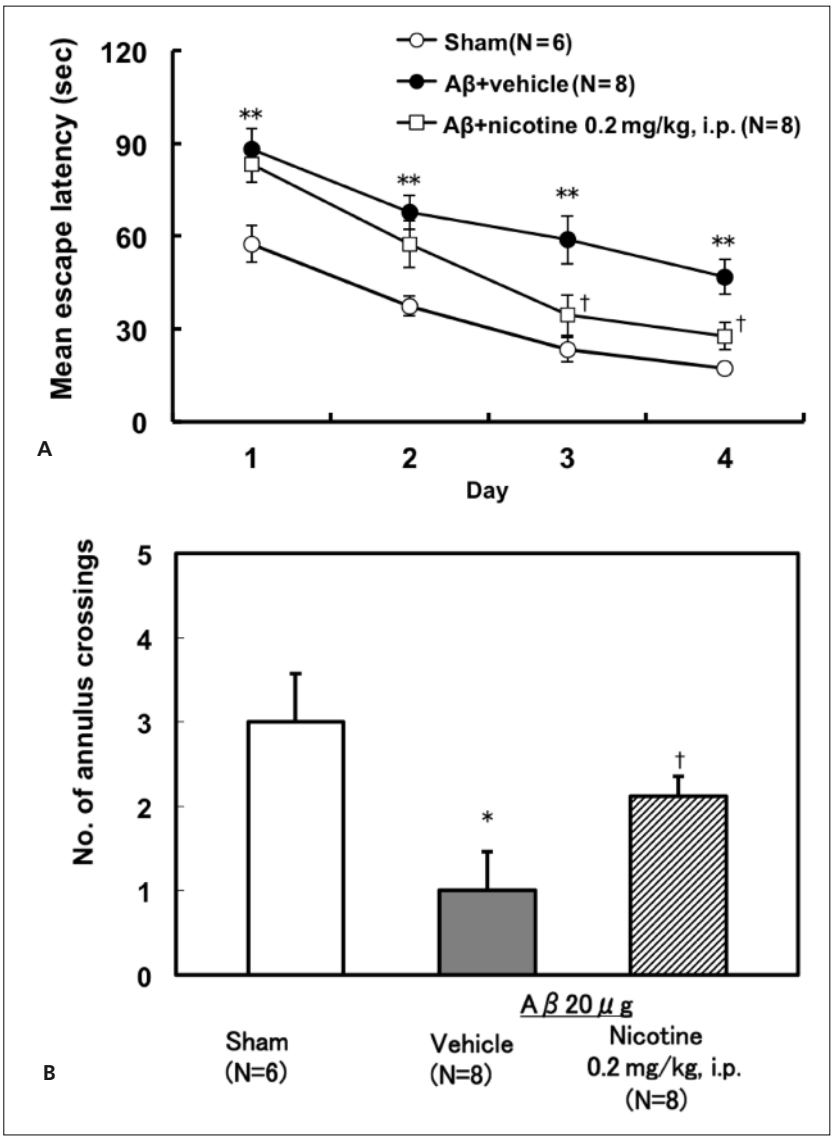

Figure 1. Effect of nicotine on learning and memory deficit in Morris water maze performance in rat cognitive dysfunction model induced by intracerebroventricular infusion of $A\beta_{1\text{-}42}$. $A\beta_{1\text{-}42}$ (20 μg) was continuously infused into right ventricle for 3 days by attachment of an infusion kit to an osmotic mini-pump. Nicotine (0.2 mg/kg, i.p.) was administered once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$ infusion until the last day of behavioral assessment. In water maze tasks, acquisition trials ***(A)*** were performed once a day for 4 days (i.e. the days 87-90 after the start of $A\beta_{1\text{-}42}$ infusion), whereas a probe trial ***(B)*** was conducted for 60 s at 2 h after the final acquisition test. Data represents the mean ± SEM. Number of rats used is indicated in parentheses. $*p < 0.05$, $**p < 0.01$ vs sham, $\dagger p < 0.05$ vs vehicle (Student *t*-test).

1B). Nicotine treatment showed a significant inhibition in the decline in the number of annulus crossing (Figure 1B), suggesting improvement of memory deficit.

Effect of Nicotine on Learning and Memory Deficit In Passive Avoidance Task in Rat Cognitive Dysfunction Model Induced by Intracerebroventricular Infusion of $A\beta_{1\text{-}42}$

Figure 2 shows the effects of nicotine on learning and memory deficits in passive avoidance task with a rat cognitive dysfunction model induced by intracerebroventricular infusion of $A\beta_{1\text{-}42}$. The acquisition trial and retention trial were performed 80 and 84 days after the start of $A\beta_{1\text{-}42}$ infusion, respectively. In the acquisition trial, intracerebroventricular infusion of $A\beta_{1\text{-}42}$ did not affect the number of shocks at all (Figure 2A). Also, administration of nicotine (0.2 mg/kg, once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$ infusion) showed little effect on the number of shocks. In the retention trial, step-through latency was reduced by the infusion of $A\beta_{1\text{-}42}$, while statistical significance was not detected in the reduction (Figure 2B, $p = 0.28$). The treatment with nicotine ameliorated the $A\beta_{1\text{-}42}$-induced decrease in step-through latency, but the effect was not statistically significant in this case as well (Figure 2B, $p = 0.11$).

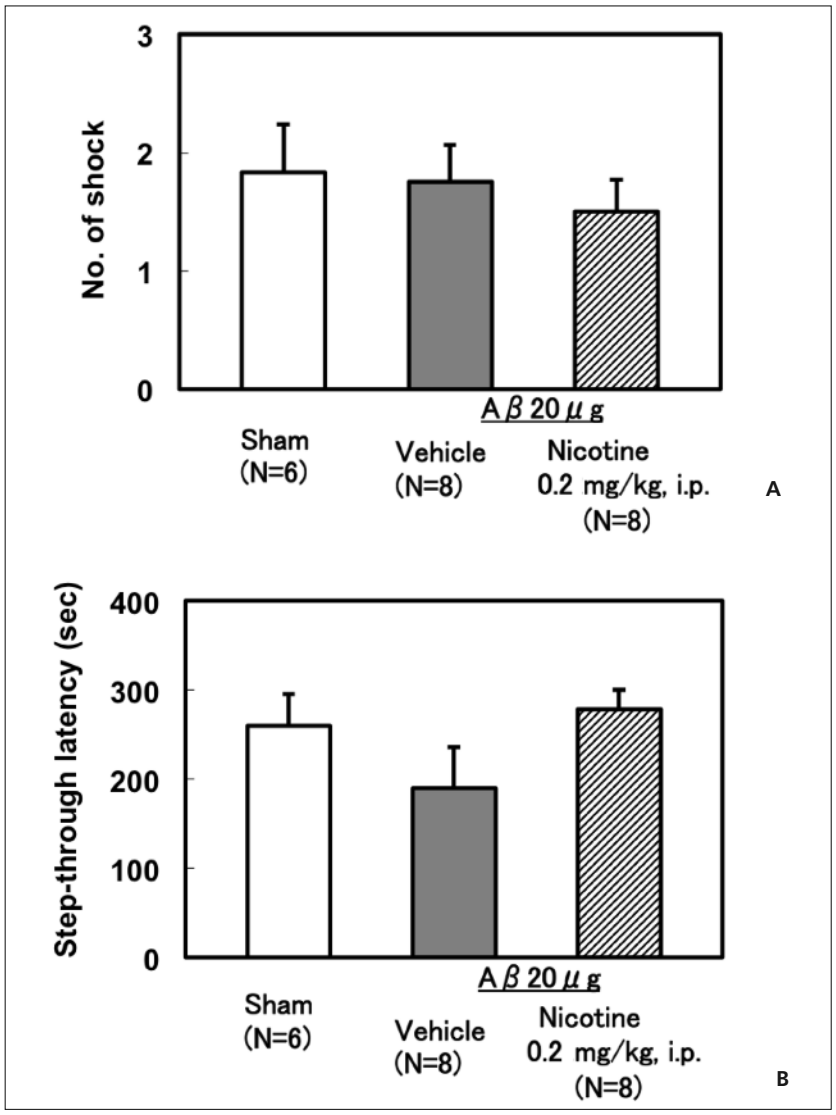

Figure 2. Effect of nicotine on learning and memory deficit in passive avoidance performance in rat cognitive dysfunction model induced by intracerebroventricular infusion of $A\beta_{1\text{-}42}$. $A\beta_{1\text{-}42}$ (20 μg) was continuously infused into right ventricle for 3 days by attachment of an infusion kit to an osmotic mini-pump. Nicotine (0.2 mg/kg, i.p.) was administered once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$ infusion until the last day of behavioral assessment. The acquisition and retention trials were performed 80 days and 84 days after the start of $A\beta_{1\text{-}42}$ infusion, respectively. ***A,*** Number of shocks during an acquisition trial. ***B,*** Step-through latency during a retention trial. Each column represents the mean ± SEM. Numbers of rats used are shown in parentheses.

Effect of Nicotine on Chat Activity in Various Regions of the Brain in Rat Cognitive Dysfunction Model Induced by Intracerebroventricular Infusion of $A\beta_{1\text{-}42}$

The level of ChAT activity, a marker of cholinergic neuron, has extensively been examined in various regions of the brain in a variety of animal models. Figure 3 shows the levels of ChAT of the rats following the completion of water maze test on day 90. Intracerebroventricular infusion of $A\beta_{1\text{-}42}$ significantly decreased the level of ChAT activity in posterior cortex and striatum (Figure 3 B, D), but not in anterior cortex or hippocampus (Figure 3A, C). Repeated administration of nicotine (0.2 mg/kg, once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$ infusion) did not reverse the level of ChAT in posterior cortex and striatum (Figure 3B, D). The nicotine treatment did not show any effect on the ChAT activity in anterior cortex or hippocampus (Figure 3A, C).

Effect of Nicotine on HC3 binding in Various Regions of The Brain in Rat Cognitive Dysfunction Model Induced by Intracerebroventricular Infusion of $A\beta_{1\text{-}42}$

Figure 4 shows the effect of nicotine on HC-3 binding, a marker of presynaptic cholinergic nerve terminals, which was measured after water maze test on day 90. As shown in Figure 4, intracerebroventricular infusion of $A\beta_{1\text{-}42}$ significantly decreased the level of HC-3 binding in anterior cortex, posterior cortex, and hippocampus

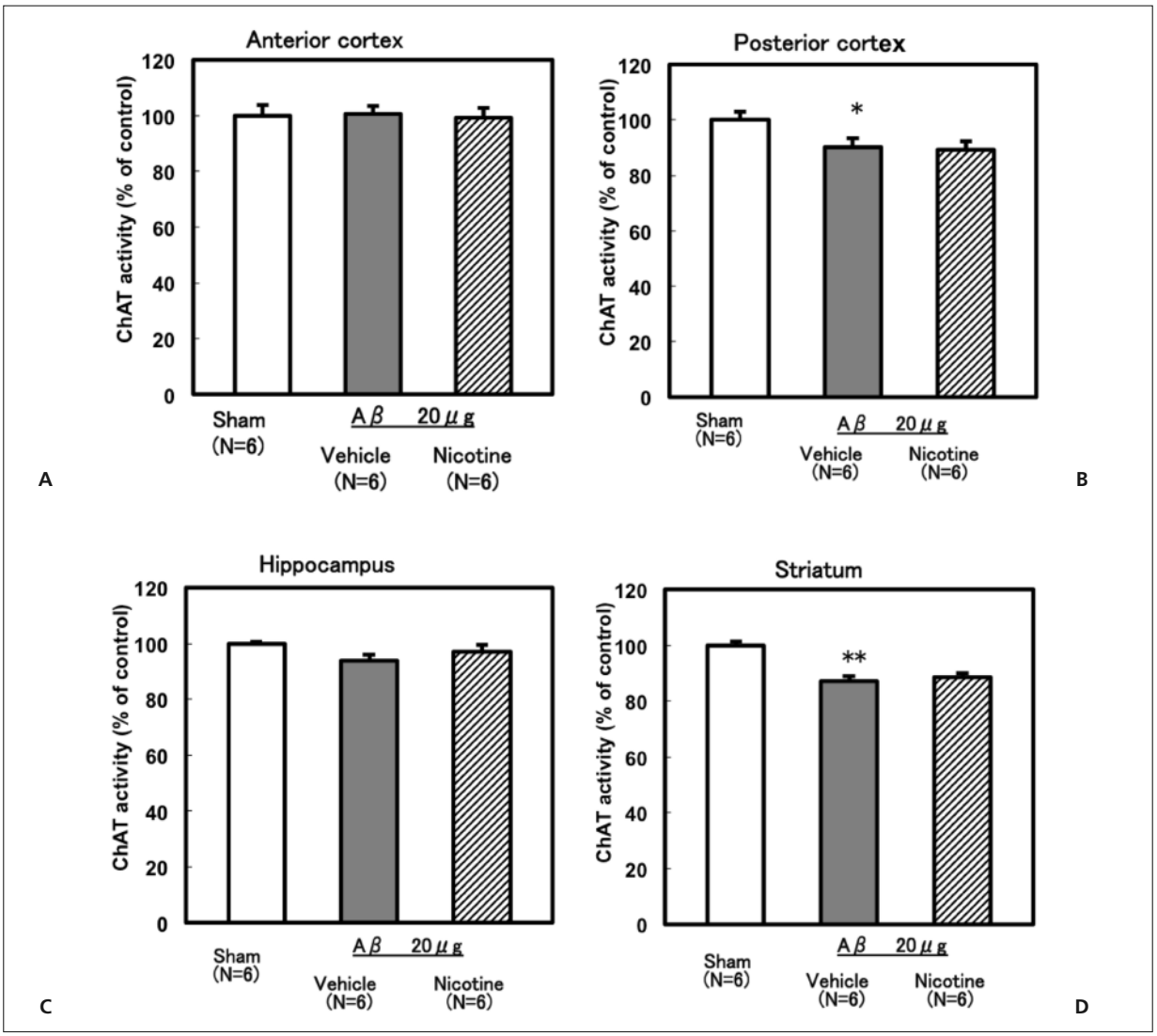

Figure 3. Effect of nicotine on ChAT activity in various regions of the brain in rat cognitive dysfunction model induced by intracerebroventricular infusion of $A\beta_{1\text{-}42}$. $A\beta_{1\text{-}42}$ (20 μg) was continuously infused into right ventricle for 3 days by attachment of an infusion kit to an osmotic mini-pump. Nicotine (0.2 mg/kg, i.p.) was administered once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$ infusion until the last day of behavioral assessment. On the day 90, after the learning and memory studies of water maze tasks were completed, ChAT activity in anterior cortex ***(A)***, posterior cortex ***(B)***, hippocampus ***(C)*** and striatum ***(D)*** were measured as described in Materials and Methods. The data represents the mean ± SEM. Number of rats used is shown in parentheses. $*p < 0.05$, $**p < 0.01$ vs sham (Student *t*-test).

(Figure 4 A-C), but not in striatum (Figure 4 D). Administration of nicotine (0.2 mg/kg, once a day, 5 days a week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$) significantly inhibited the decrease in HC-3 binding in anterior cortex and hippocampus (Figure 4 A, C). The effect of nicotine was also observed in posterior cortex, which was not statistically significant (Figure 4 B). Nicotine showed little effect on the HC-3 binding in striatum (Figure 4 D).

Discussion

The data in the present study have shown that nicotine improved learning and memory deficit in Morris water maze performance in a rat cognitive dysfunction model induced by intracerebroventricular infusion of Aβ. Consistent with our study, there are several reports, which have shown an improvement of episodic and working memories by nicotine or nicotine agonists using

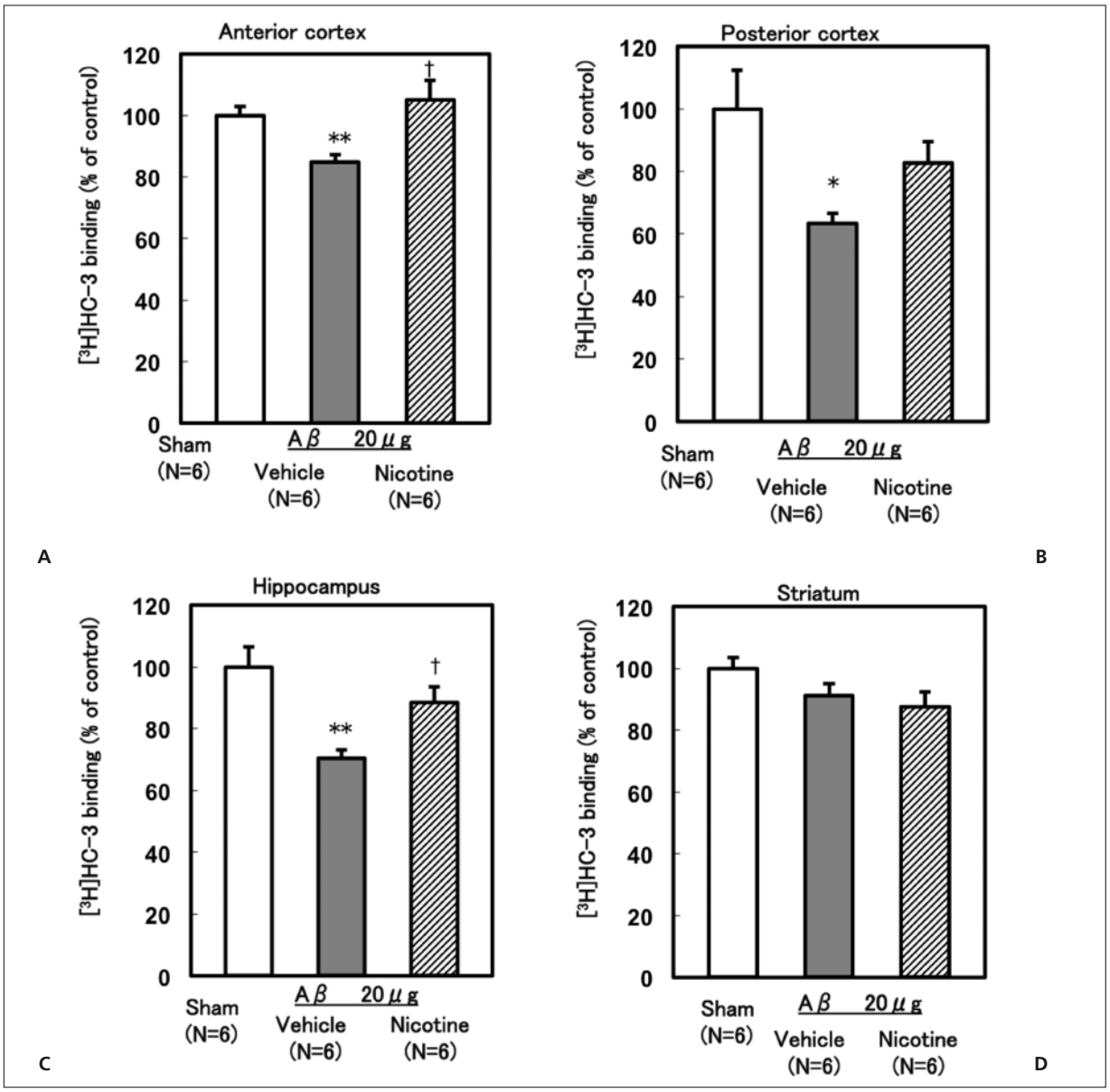

Figure 4. Effect of nicotine on HC3 binding in various regions of the brain in rat cognitive dysfunction model induced by intracerebroventricular infusion of $A\beta_{1\text{-}42}$. $A\beta_{1\text{-}42}$ (20 μg) was continuously infused into right ventricle for 3 days by attachment of an infusion kit to an osmotic mini-pump. Nicotine (0.2 mg/kg, i.p.) was administered once a day, 5 days per week, beginning 3 weeks after the start of $A\beta_{1\text{-}42}$ infusion until the last day of behavioral assessment. On the day 90, after the learning and memory studies of water maze tasks were completed, HC3 binding in anterior cortex ***(A)***, posterior cortex ***(B)***, hippocampus ***(C)*** and striatum ***(D)*** were measured as described in Materials and Methods. The data represents the mean ± S.E.M. Number of rats used is shown in parentheses. *$p < 0.05$, **$p < 0.01$ vs sham, †$p < 0.05$ vs vehicle (Student *t*-test).

animal models. For example, treatment with nicotine for 6 weeks prevented impairment of learning and short-term memory in radial arm water maze test in a rat model of AD induced by 14-day intracerebroventricular infusion of Aβ peptides[25]. In addition, Boess et al[26] have reported that an a7nAChR agonist improves working memory in water maze test in aged rats. These findings including ours indicate the potential of nicotine and other nAChR agonists as agents for neurodegenerative diseases including AD.

In the animal model used in our study, the Aβ infusion decreased HC-3 binding sites in the brain and the nicotine treatment significantly inhibited the decrease in anterior cortex and hippocampus. In contrast, nicotine failed to show

substantial effect on the Aβ-induced reduction in the level of ChAT, a marker for ACh terminals. HC-3 is a marker for choline uptake sites, and the reuptake of choline is the rate limiting step in the synthesis of acetylcholine[24]. Thus, our data indicates that nicotine improves the Aβ-induced decrease of cholinergic function in anterior cortex and hippocampus without affecting the number of ACh terminals, and leads to functional restoration on Aβ-induced spatial cognitive deficits. Further studies are needed to elucidate the mechanism underlying the increase in the HC-3 binding by nicotine.

The changes of the two markers, ChAT and HC-3 binding, were different in our study, while both are expressed in the cholinergic terminal. In this regard, it should be noted that non-toxic submicromolar concentration of Aβ impairs HC-3 sensitive high-affinity choline uptake in a cholinergic cell line NG108-15 through protein kinase C signaling[27], suggesting the selective effect for HC-3 binding. Nicotine increased the HC-3 binding in the Aβ-treated but not the untreated rats. Thus, the mechanisms for the effect of nicotine on HC-binding might link to those for the Aβ-induced reduction of HC-3 binding, which could explain the different effect of nicotine on ChAT and HC-3. Another interest in the study in ChAT and HC-3 binding is that the changes in the two parameters show different patterns in the brain areas, but the reason of these differences is unclear at this point.

The mechanisms by which nicotine treatment improves learning and memory deficits have not been suggested other than the relation to HC-3 in the present study, but the effect might be explained by a direct interaction between Aβ and nAChRs, such as α7 and α4β2[14,28]. Aβ interacts with a7 nAChR with an affinity of picomolar levels, while the affinity of Aβ to a4β2 nAChR is 100-5,000 times lower. On the other hand, there are conflicting data concerning the functional effect of Aβ on nAChRs, i.e. some studies show the activation of the receptor, whereas others report inhibitory action[14,28], suggesting that the action of Aβ on nAChRs might be dependent on species of the peptide and/or incubation times. It is, therefore, possible that nicotine treatment might modulate the interaction between Aβ and nAChRs, though the concentration and species of Aβ in the brain is not known in our study.

Another explanation could be an involvement of neurotransmitter release stimulated by nicotine. Numerous studies have shown that nAChRs are located on presynaptic, preterminal sites and interact with other metabolic or ionotrophic receptors[29,30] and modulate release of various neurotransmitters[31-38] including glutamate, γ-aminobutyric acid (GABA), dopamine, noradrenaline and glycine via pre-synaptic sites of action and the release of these neurotransmitters are likely essential steps in mediating the cognitive enhancing effects of nicotine[39,40].

Conclusions

Repeated treatment of nicotine improved learning and memory deficit in a rat cognitive dysfunction model induced by intracerebroventricular infusion of Aβ, suggesting the potential of nicotine as a therapeutic drug for AD. Although systemic use of nicotine is problematic due to its peripheral effects and safety concerns[41,42], several nAChR ligands are being developed targeting wide range of disorders. This study supports the potential usefulness of nAChR ligands for the treatment of AD through the enhancement of cholinergic neuronal function.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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