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Effects of amyloid-β-(25–35) on passive avoidance, radial-arm maze learning and choline acetyltransferase activity in the rat

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Abstract

To investigate the neurotoxicity of amyloid-β-(25–35), which is thought to be the active site of amyloid-β, the peptide was injected into the lateral ventricle of rats. A single intracerebroventricular (i.c.v.) injection of amyloid-β-(25–35) at a dose of 15 nmol/rat induced a marked decrease in latency in step-through passive avoidance task. Amyloid-β-(35–25), reverse sequence of amyloid-β-(25–35), was without harmful effects on passive avoidance performance. The amyloid-β-(25–35) at a dose of 5 or 15 nmol/rat impaired radial-arm maze performance, and induced a decrease in choline acetyltransferase activity in the medial septum, cortex and hippocampus, but not in the basal forebrain. The number of choline acetyltransferase-immunoreactive cells in the medial septum was decreased, in conformity with the decrease in choline acetyltransferase activity of the area. These results suggest that learning and cognitive disturbance induced by i.c.v. injection of amyloid-β-(25–35) is associated with the dysfunction of cholinergic neuronal system in the brain.

Keywords: Choline acetyltransferase; Amyloid-β; Passive avoidance; Maze learning; (Rat)

1. Introduction

Alzheimer's disease is characterized by the presence of senile plaques, reactive astrocytes and neurofibrillary tangles, and synaptic and neuronal loss in several areas of the brain. The principal protein component of the plaques is 39- to 43-amino acid amyloid peptide, derived from a larger amyloid precursor protein (Glenner and Wong, 1984; Masters et al., 1985). It has been suggested that the accumulation of amyloid-β in the brain plays a crucial role in the pathogenesis of Alzheimer's disease (Selkoe, 1991). Positive correlation of amyloid precursor protein levels in the neocortex and hippocampus with escape latency in a water maze task was found in rats by Lin et al. (1999). In their study, the cognitive changes caused by cholinergic lesions were reversed by muscarinic receptor agonist, which was also effective in decreasing amyloid precursor protein levels in tissues. Among the neurochemical parameters described for the brain of Alzheimer's disease patients, the decrease in the choline acetyltransferase activity is the most prominent and serves as an excellent biochemical correlate on the severity of Alzheimer's disease dementia (Bartus et al., 1982; Koshimura et al., 1986; Bierer et al., 1995). These findings support the hypothesis that cholinergic neurons are particularly vulnerable in Alzheimer's disease brain (Whitehouse et al., 1982) and that cholinergic function is important in the learning and memory processes (Dutar et al., 1995).

Pedersen et al. (1996) reported that amyloid-β-(1–28), amyloid-β-(25–35), and amyloid-β-(1–42) reduced the choline acetyltransferase activity and intracellular concentration of acetylcholine in a cell line that expresses cholinergic characteristics (SN56; generated by the fusion of neuroblastoma cells with primary mouse septal neurons). In vivo experiments by Nitta et al. (1994) demonstrated that chronic infusion of amyloid-β-(1–40) into the cerebral ventricle for 14 days by using mini-osmotic pump caused performance impairment in the water maze learning and decrease in the choline acetyltransferase activity in the frontal cortex and hippocampus in the rat. Moreover,

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Harkany et al. (1999) found that a single injection of amyloid-β-(1–42) into the right magnocellular nucleus basalis caused impairment of passive avoidance learning and reduction in the choline acetyltransferase activity in the cerebral cortex. However, in primary cultures of the rat septum the choline acetyltransferase activity was not reduced following a 12-h treatment with solubilized amyloid-β-(1–42) (Hoshi et al., 1997). Kar et al. (1998) likewise demonstrated that the choline acetyltransferase activity was not altered by 1 or 2 h incubation with amyloid-β-(1–40) in tissue homogenates or slice preparations of the rat striatum, cortex and hippocampus. Moreover, Sigurdsson et al. (1997) reported that bilateral injections of amyloid-β-(25–35) into the amygdala of rats induced histopathological changes like the appearance of reactive astrocytes and neuronal shrinkage, but that it failed to cause any disturbance in the Morris water maze performance or in the one-way conditioned avoidance response, and that no influence of the amyloid-β-(25–35) injections was observed in amygdaloid choline acetyltransferase activity. They suggested that amyloid-β-induced histopathological changes were not associated with the reduction of cholinergic terminals projecting from the magnocellular nucleus basalis. Moreover, repeated intracerebroventricular (i.c.v.) injections of amyloid-β-(25–35) for 7 days failed to modify choline acetyltransferase activity in the cerebral cortex (Pavia et al., 2000). Thus, the results on the effects of amyloid-β on choline acetyltransferase activity and cognitive function are variable, perhaps due to the differences in the sites of administration and experimental models. In the present study, the effect of a single i.c.v. injection of amyloid-β-(25–35), in amount sufficient to induce neurotoxicity in neuronal cultures (Pike et al., 1993, 1995), was examined whether cognitive deficits could be induced by this direct injection, using passive avoidance and radial-arm maze tasks. Furthermore, we studied whether amyloid-β-(25–35) injections affect choline acetyltransferase activity and choline acetyltransferase-immunoreactivity in discrete areas of the brain.

2. Materials and methods

2.1. Animals

All animal care and treatments were conducted in accordance with the guidelines of the animal use and care committee of the Research Laboratory, Zenyaku Kogyo, in conformity to the NIH Guide for the Care and Use of Laboratory Animals (1978), and were approved by the above committee.

Ninety-five male rats of the Sprague–Dawley strain (Charles River, Kanagawa, Japan) at 7 weeks of age (200–260 g body weight) were used in the experiments. Separate groups of rats were used in each experiment except for the measurement of choline acetyltransferase activity where the animals were those used for the experiment of passive avoidance task. Rats were housed in a cage in a group of 4 to 6 rats, in a room maintained at around 22°C with a 12-h light/dark cycle. However, in the radial-arm maze study, rats were kept individually at the beginning of pretraining.

2.2. Peptide injection

Amyloid-β-(25–35) (H-GSNKGAIIGLM-OH, Peptide Institute, Osaka, Japan) was dissolved in distilled water, which favors aggregation (Pike et al., 1995), at the concentration of 5 or 15 nmol/5 μl. Reverse sequence of amyloid-β-(25–35) (amyloid-β-(35–25), H-MLGIIAGKNSG-OH, Sigma, St. Louis, MO, USA) was used as a reference peptide. It was dissolved in distilled water at the concentration of 15 nmol/5 μl. These solutions were incubated at 37°C for 4 days before use, since this procedure is known to produce insoluble precipitates and markedly facilitated the appearance of learning deficits in several tasks (Maurice et al., 1996; Delobette et al., 1997). I.c.v. injection was performed under pentobarbital sodium (50 mg/kg) anesthesia by means of a stereotaxic apparatus. Referring to the atlas of Paxinos and Watson (1982), injection cannula was inserted stereotaxically at a site 0.8 mm posterior and 1.5 mm lateral to the bregma and 4.0 mm below the surface of the cranium to place the tip of the cannula in the left lateral ventricle. Five μl of amyloid-β-(25–35) or vehicle was injected with Hamilton syringe. After surgery, the animals were returned to their home cages. The injection site was checked by injecting trypan blue in stead of peptide in preliminary experiments.

2.3. Passive avoidance task

The passive avoidance test was started 4 days after the injection of amyloid-β-(25–35). The apparatus (Neuroscience, Tokyo, Japan) consisted of small illuminated chamber connected with a larger dark chamber. Two chambers were separated by a guillotine door. On the first and second days of testing, each rat was placed in the apparatus and left for 3 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. Immediately after entering the dark chamber, the door was closed and an inescapable scrambled electric shock (100 V, 0.3 mA, 0.5 s once) was delivered through the floor grid. Then the rats were returned to their home cages. Twenty-four hours later, each rat was again placed in the illuminated chamber (retention trial). The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as latency in both acquisition and retention trials (maximum 300 s).

The flinch, vocalization and jump thresholds with electric footshock were measured, 8 days after the injection of amyloid-β-(25–35) at the dose of 15 nmol/5 μl. Each rat

Table 1
Effects of amyloid-β-(25–35) on the flinch, vocalization and jump thresholds with electric footshock in rats

	n	Threshold (mA)		
		Flinch	Vocalization	Jump
Vehicle	8	0.31 ± 0.03	0.41 ± 0.03	0.73 ± 0.08
Amyloid-β-(25–35)	8	0.30 ± 0.03	0.39 ± 0.04	0.75 ± 0.07

Each value shows the mean ± S.E.M.

was placed on the grid floor and scrambled electric footshock was delivered. The shock intensity was raised stepwise manually from 0.1 to 1.0 mA (at 0.1 mA intervals; shock duration: 0.5 s; inter-shock interval: 5 s) until a flinch, vocalization and jump were observed. The shock at 0.3 mA was the threshold for flinch, but subthresholds for vocalization and jump (Table 1).

2.4. Radial-arm maze performance

Each rat was tested in a standard radial-arm maze (Muromachi Kikai, Tokyo, Japan) that was a slightly modified version of the one originally used by Olton and Samuelson (1976). Details of apparatus have been described in a previous paper (Yamaguchi and Kobayashi, 1994). A restricted feeding schedule and pretraining began 6 days after the i.c.v. injection of amyloid-β-(25–35). Body weights at the beginning of test trials (11 days after the amyloid-β injection) were reduced to about 80% of the time of pretraining by reduction of the daily ration of food (CE-7, Clea Japan, Tokyo, Japan). Subsequently, the body weight of each rat was increased at 5 g per week by manipulation of the supply of food. In order to habituate the rats to the maze, they were pretrained once a day for 3 days. On the first day of pretraining, rats were placed in groups of six rats in the maze without any reward bait (crystallized sugar) and left explore the arms for 10 min. On the second and third days of pretraining, rats of the same groups were placed in the maze with bait scattered on the platform and arms including food cups, and left for 10 min. The test trials started 3 days after pretraining and were carried out for 15 days. In each trial, a rat was placed on the central hub of the maze and allowed to visit the wells at the end of each of the eight arms, each of which had been baited one sugar crystal. Running paths were tracked with a camera fixed on the ceiling of the room and stored in a computer (BTA-2 system, Muromachi Kikai). Each trial was continued until the bait of all eight wells had been consumed, or until 16 choices had been made, or until 10 min had elapsed, whichever occurred first. An interval of 3 min was inserted between the fourth and fifth choices. This procedure is thought to be a more sensitive assessment of short-term memory for previous choices due to the increase in cognitive load added by the interval (Sweeney et al., 1997). During interval-interposition, the rat was returned to its home cage. Rats were required to learn how to obtain bait in the well and remember not to re-enter those arms that had been visited. Re-entry into the arm that had been visited before was scored as an error. The number of consecutive correct choices prior to re-entry into a previously visited arm (the number of initial correct responses) and the running speed were recorded as indices of performance. For the analysis of performance, 15 test trials were divided into the following five blocks, each of which consisted of three trials: block 1, trials 1–3; block 2, trials 4–6; block 3, trials 7–9; block 4, trials 10–12; block 5, trials 13–15. The mean values and standard errors of the three trials in each block were calculated.

2.5. Choline acetyltransferase activity measurement

Choline acetyltransferase activity was assayed as described by Fonnum (1975), 8 days after the injection of amyloid-β-(25–35) (1 day after passive avoidance tests). Rats were killed by decapitation and brains were quickly removed and placed on an ice-cooled glass plate. The brain was placed on its dorsal surface and a coronal section was made through the optic chiasm. The medial septum, readily visible and situated between the lateral ventricles, was pinched out with fine dissecting forceps. The basal forebrain, the region directly ventral to the anterior commisure containing the nucleus basalis, was then removed from the remaining anterior portion of the brain. A piece of the cerebral cortex was dissected out at the motor area of the frontoparietal cortex. Then, the hippocampus was dissected out and a piece of dorsal hippocampus was separated. These tissues were homogenized in 1.5 ml tubes containing 400 μl of cold 50 mM phosphate buffer (pH 6.8) with 10 mM EDTA and 0.5% Triton X-100. The tubes were centrifuged at $20{,}000 \times g$ for 5 min at 4°C, and the supernatants were used as the enzyme solution. Each enzyme solution was incubated at 37°C in a medium containing radiolabelled [^{14}C] acetyl coenzyme A (Amersham, Tokyo, Japan) to produce acetylcholine, acetyl coenzyme A being combined with choline in the medium by the action of choline acetyltransferase. The amount of radioactive acetylcholine in each tube was measured for 5 min in a scintillation counter. Protein concentration of the sample in each tube was measured by the method of Bradford (1976) with a Protein Assay Kit I (Bio-Rad Laboratories, CA, USA) with bovine gamma globulin as a standard. Choline acetyltransferase activity is expressed as nmol acetylcholine synthesized per mg protein per h.

2.6. Immunohistochemistry

After the i.c.v. injection of amyloid-β-(25–35) at a dose of 5 or 15 nmol/rat in 5 μl as stated in Section 2.2, the animals were returned to their home cages. Eight days later, the rats were deeply anaesthetized with sodium

pentobarbital and perfused through the ascending aorta with 50 ml of saline solution followed by 200–250 ml of ice-cold paraformaldehyde solution (4% in phosphate-buffer, pH 7.4). The brains were removed, postfixed in the same fixative for 4 h, cryoprotected in sucrose solution (18% in phosphate-buffer) for at least 24 h at 4°C, and coronal sections 20 mm-thick were cut in a cryostat at the level of the septum. The sections were mounted on gelatin-coated slides, and allowed to dry at room temperature.

The sections were immunostained by the streptavidin–biotin method with a commercial Kit (Histofine SAB-PO, Nichirei, Tokyo, Japan). After 1 h of preincubation at room temperature in 10% normal goat serum in phosphate-buffered saline (PBS) and 0.3% Triton-X-100, the slides were incubated overnight at room temperature with the choline acetyltransferase-specific antibody (Chemicon, CA, USA) at a dilution of 1: 1000 in PBS. On the following day, the sections were washed in PBS and incubated for 1 h at room temperature with biotinylated anti-rabbit immunoglobulin (Ig)G (Nichirei Kit, Tokyo, Japan). After a further PBS wash, incubation with the peroxidase-conjugated streptavidin was followed for 1 h at room temperature. For the peroxidase reaction, the sections were immersed in 0.02% solution of 3,3′-diaminobenzidine in 0.05 M Tris–HCl buffer (pH 7.6) that contained 0.005% H_2O_2 for 10–15 min at room temperature. Then the sections were washed in distilled water for 5 min, dehydrated through an ethanol series, cleared in xylene and mounted. Negative control slides were incubated overnight without primary antibody and run through the entire procedures.

For microscopic analysis, cell counting was performed under a $10\times$ objective using a calibrated eyepiece grid. The choline acetyltransferase-immunoreactive cells were identified by purple-black deposits in the cytoplasm. Four sections were selected in each animal. The sections were selected one each from consecutive 5th–7th ones of serial coronal sections. The total number of choline acetyltransferase-positive cells in the four sections was counted in the region of the medial septum.

2.7. Statistical analysis

Results were expressed as mean ± S.E.M. The data of passive avoidance were analyzed using the Kruskal–Wallis analysis of variances by ranks, which was followed by the non-parametric Dunn's multiple comparison test. In the data of flinch, vocalization and jump thresholds, statistical comparisons were made with Mann–Whitney *U*-test. In the data of radial-arm maze, choline acetyltransferase activity and the number of choline acetyltransferase-positive cells, the data showed normal distribution, and the statistical significance of differences between groups was calculated by two-way repeated analysis of variance (ANOVA) or one-way ANOVA, which was followed by Dunnett's multiple comparison test. The criterion for significance was $P < 0.05$ in all the analyses.

3. Results

3.1. Passive avoidance task

Since we found no difference between vehicle-treated control rats and reverse peptide amyloid-β-(35–25) at dose of 15 nmol/rat in the acquisition and retention trials (Fig. 1A), all the following experiments were carried out comparing between vehicle-treated control rats and amyloid-β-(25–35)-treated rats. In the acquisition trial (6 days after the operation), we found no difference between the vehicle-injected control and amyloid-β-(25–35)-treated rats in the step-through latency on acquisition trials (Fig. 1B). The results of Kruskal–Wallis test was $H = 0.439$, $P > 0.05$. Therefore, the analgesia 6 days after the operation was not strong enough to induce any effect on passive avoidance tasks. However, the i.c.v. administration of amyloid-β-(25–35) reduced step-through latency in the retention trials (Fig. 1B). The results of the Kruskal–Wallis test was $H = 12.58$, $P < 0.01$. Dunn's multiple range test revealed that amyloid-β-(25–35) at the dose of 15 nmol significantly reduced the step-through latency ($P < 0.01$). As changes in the responses to electric footshock in

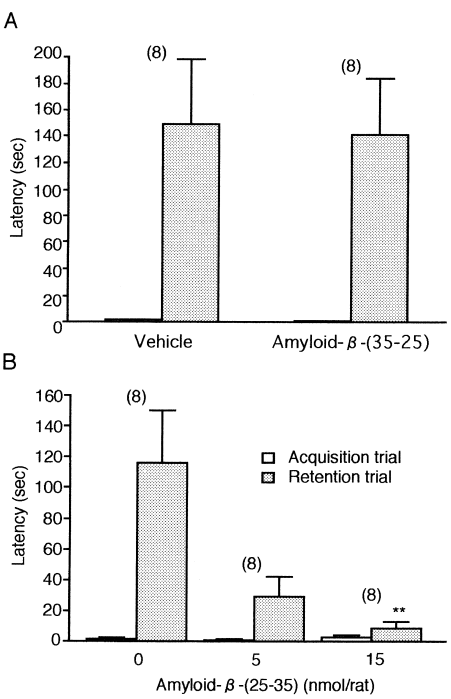

Fig. 1. Effects of i.c.v. injection of amyloid-β-(35–25) (15 nmol/rat, reverse sequence of amyloid-β-(25–35))(A), and amyloid-β-(25–35) (5 or 15 nmol/rat)(B) on passive avoidance task. Each column represents the mean latency and vertical bars show S.E.M. The number in parentheses indicates the number of animals. $^{**}P < 0.01$, compared with vehicle-treated control rats (Dunn's multiple range test).

the acquisition trial could influence passive avoidance learning, we examined the effect of amyloid-β on the response to electric footshock. As shown in Table 1, i.c.v. injection of amyloid-β-(25–35) at dose of 15 nmol/rat did not alter the flinch, vocalization and jump thresholds significantly ($P > 0.05$, Mann–Whitney U-test), showing no changes in the footshock sensitivity by amyloid-β-(25–35) treatment. Thus, amyloid-β-(25–35)-treated rats exhibited decreased retention of passive avoidance memory.

3.2. Radial-arm maze performance

The rats given i.c.v. injection of amyloid-β-(25–35) showed a significantly smaller number of initial correct responses compared with vehicle-injected control rats (Fig. 2A). A two-way repeated ANOVA revealed significant effects of injection of amyloid-β-(25–35) [$F(2,105) = 70.59$, $P < 0.01$] and trials [$F(4,105) = 8.61$, $P < 0.01$] and no significant effect of interaction [$F(2,105) = 0.99$, $P > 0.05$]. In the trials 2–4 at the dose of 5 nmol/rat and trials 1–5 at the dose of 15 nmol/rat, the initial correct responses were reduced compared with matched vehicle-injected control rats ($P < 0.01$, Dunnett's multiple comparison test). The rats given i.c.v. injection of amyloid-β-(25–35) showed a significantly larger number of total errors compared with vehicle-injected control rats (Fig. 2B). A two-way repeated ANOVA revealed significant effects of injection of amyloid-β-(25–35) [$F(2,105) = 11.36$, $P < 0.01$] and trials [$F(4,105) = 5.04$, $P < 0.05$] and no significant effect of interaction [$F(2,105) = 0.69$, $P > 0.05$]. In the trial 3 at the dose of 15 nmol/rat, the total errors were significantly increased compared with matched vehicle-injected control rats ($P < 0.01$, Dunnett's multiple comparison test). Running speed of amyloid-β-treated rats (ranged from 24.2 ± 0.7 to 30.9 ± 1.7 s for 5 nmol group, and from 23.2 ± 0.4 to 28.0 ± 1.3 s for 15 nmol group) was not significantly different from that of vehicle-injected control rats (ranged from 24.1 ± 0.5 to 32.7 to 1.6 s)[$F(2,21) = 0.98$, $P > 0.05$ on block 1; $F(2,21) = 1.08$, $P > 0.05$ on block 2, $F(2,21) = 1.14$, $P > 0.05$ on block 3; $F(2,21) = 2.34$, $P > 0.05$ on block 4; $F(2,21) = 2.32$, $P > 0.05$ on block 5]. Thus, amyloid-β-treated rats showed impaired learning in interval-interposed radial-arm maze task.

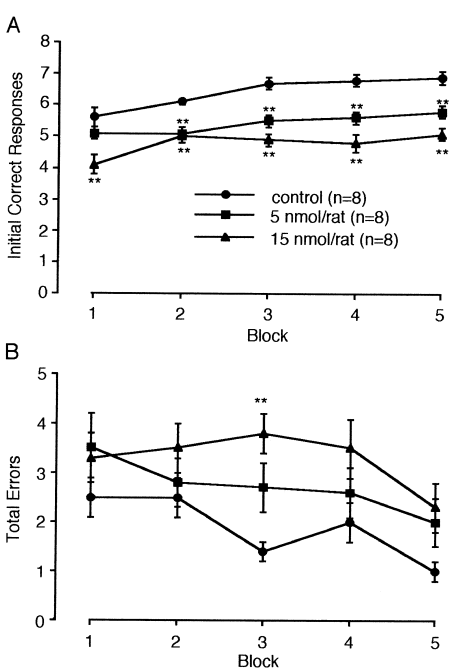

Fig. 2. Effects of i.c.v injection of amyloid-β-(25–35) on radial-arm maze performance. Each value with standard errors represents the mean number of initial correct responses (A) and total errors (B) in each block of consecutive three trials. $^{**}P < 0.01$, compared with vehicle-treated control rats (Dunnett's multiple comparison test).

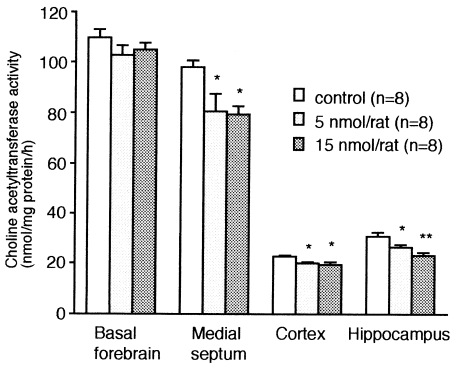

Fig. 3. Effects of i.c.v. injection of amyloid-β-(25–35) on choline acetyltransferase activity in four brain regions of rats. Choline acetyltransferase activity is expressed as nmol acetylcholine synthesized per mg protein per h. $^{**}P < 0.01$, $^{*}P < 0.05$, compared with vehicle-treated controls (Dunnett's multiple comparison test).

3.3. Choline acetyltransferase activity

Choline acetyltransferase activities in the basal forebrain containing the nucleus basalis, medial septum, cortex

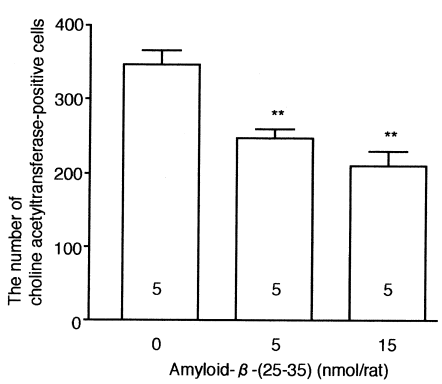

Fig. 4. Effect of i.c.v. injection of amyloid-β-(25–35) on the number of choline acetyltransferase-immunoreactive cells in the medial septum in a total of four sections. The number in each column indicates the number of animals. $^{**}P < 0.01$, compared with vehicle-treated control (Dunnett's multiple comparison test).

and hippocampus of the vehicle-treated rats was 109.9 ± 3.4, 98.0 ± 3.4, 23.0 ± 0.7 and 30.9 ± 1.5 nmol/mg protein/h, respectively. Choline acetyltransferase activities were decreased by i.c.v. injection of amyloid-β in the medial septum [$F(2, 21) = 4.78$, $P < 0.05$], cortex [$F(2,21) = 4.25$, $P < 0.05$] and hippocampus [$F(2,21) = 4.76$, $P < 0.01$], but not in the basal forebrain [$F(2,21) = 1.23$, $P > 0.05$] as compared with vehicle-injected controls (Fig. 3). In the medial septum and cortex, administration of amyloid-β-(25–35) at the dose of 5 or 15 nmol/rat significantly decreased choline acetyltransferase activity compared with matched vehicle-injected control rats ($P < 0.05$, Dunnett's multiple comparison test). In the hippocampus, administration of amyloid-β-(25–35) also reduced choline acetyltransferase activity at the dose of 5 ($P < 0.05$, Dunnett's multiple comparison test) and 15 nmol/rat ($P < 0.01$, Dunnett's multiple comparison test). There were no significant differences between the two doses.

3.4. Choline acetyltransferase immunohistochemistry

The number of choline acetyltransferase-immunopositive cells in the medial septum is shown in Fig. 4. A significant reduction was found in the amyloid-β-treated groups as compared with vehicle-injected control group [$F(2, 12) = 15.28$, $P < 0.01$]. Amyloid-β-(25–35) at the dose of 5 or 15 nmol/rat significantly decreased the choline acetyltransferase-immunoreactive cells ($P < 0.01$, Dunnett's multiple comparison test). Shrinkage of perikarya was evident in several choline acetyltransferase-immunoreactive cells in the medial septum of amyloid-β-treated rats (Fig. 5).

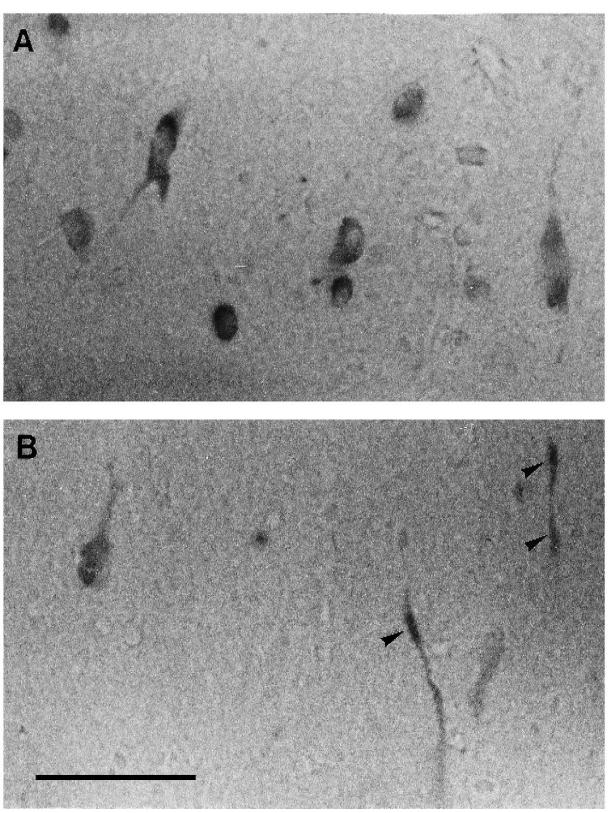

Fig. 5. Choline acetyltransferase-immunoreactive cells in the medial septum. (A) Vehicle-injected control and (B) amyloid-β-(25–35)-injected rats. Note that the amyloid-β-treated rat showed neuronal loss and perikaryal shrinkage (arrowheads). Scale bar = 50 μm.

4. Discussion

In the present study, a single i.c.v. injection of amyloid-β-(25–35) induced a significant learning disturbance in the passive avoidance and radial-arm maze tasks in the rat. Several previous studies have shown that the infusion of amyloid-β-(25–35) into the brain induced amnesia in rodents. Maurice et al. (1996) demonstrated that i.c.v. injection of amyloid-β-(25–35) induced impairments in the Y-maze, passive avoidance and water-maze tasks, and moderate neuronal loss and Congo red-stained amyloid deposits in the cortex, hippocampus and caudate. Delobette et al. (1997) confirmed the effect of i.c.v. injection of amyloid-β-(25–35) on learning disturbance in the water-maze task. Similarly, Harkany et al. (1998) demonstrated that bilateral injection of amyloid-β(Phe(SO_3H)^{24})-(25–35) in rat nucleus basalis magnocellularis induced impairment of passive-avoidance task.

It is well known that passive avoidance response can be affected by pain threshold or fear. In addition, it was also known that the lesion in the septum caused an impairment in fear condition (Gray and McNaughton, 1983) and an increase in defensiveness and attack (Albert and Chew, 1980). These findings point out the possibility that amyloid-β-(25–35)-induced impairment in passive avoidance responses was due to the change in pain threshold or fear. However, in the present study, we found no difference between vehicle-treated control rats and amyloid-β-treated rats either in the step-through latency in the acquisition trial and shock sensitivity. These results indicate that the decrease in latency by i.c.v. injection of amyloid-β-(25–35) might not be due to the changes in pain threshold or fear. These results altogether suggest that the i.c.v. injection of amyloid-β-(25–35) caused impairments in memory and learning ability.

The present study demonstrated that a single i.c.v. injection of amyloid-β-(25–35) reduced choline acetyltransferase activity in the medial septum, cortex and hippocampus. These results accord well with the findings of other investigators using full-length amyloid-β peptides showing that chronic infusion of amyloid-β-(1–40) decreased choline acetyltransferase activity in the frontal cortex and hippocampus (Nitta et al., 1994) and the injection of amyloid-β-(1–42) in the nucleus basalis reduced choline acetyltransferase activity in the cortex (Harkany et al., 1999). In this connection, the findings by Sigurdsson et

al. (1997) that bilateral injections of amyloid-β-(25–35) into the amygdala failed to cause a reduction in amygdaloid choline acetyltransferase activity appear to be at variance from so far known effects of amyloid-β-(25–35). However, their interpretation is rational in that the cholinergic termini in the amygdala are originated from the nucleus basalis that was not affected in their experiments. In the present study, the nucleus basalis also escaped from the influence of amyloid-β treatment. Further, Pavia et al. (2000) reported that i.c.v. injections of amyloid-β-(25–35) for 7 days did not modify choline acetyltransferase activity in the cerebral cortex in the rat. The reason for this discrepancy is presently unknown, but it may be related to the state of amyloid-β used. We used amyloid-β-(25–35) after 37°C incubation for 4 days, but Pavia et al. (2000) used non-incubated amyloid-β-(25–35). In support of this possibility, Maurice et al. (1996) demonstrated that incubated amyloid-β-(25–35), but not non-incubated amyloid-β-(25–35), caused impairment in passive avoidance and Y-maze tests.

Giovannelli et al. (1995) demonstrated that if amyloid-β-(25–35) was given directly on the nucleus basalis, the number of choline acetyltransferase-immunoreactive neurons was significantly decreased in the amyloid-β-(25–35)-injected side as compared to the contralateral non-injected side 1 or 2 weeks postsurgery. Similarly, Chen et al. (1996) demonstrated that injection of amyloid-β-(25–35) at a 3-nmol dosage to the hippocampus once a week for 2 weeks resulted in a marked decrease in choline acetyltransferase immunoreactivity in the medial septum. We found that the number of choline acetyltransferase-immunoreactive cells in the medial septum was decreased by a single i.c.v. injection of amyloid-β-(25–35). These findings indicate that the decrease in choline acetyltransferase activity was due to the decrease in the number of cholinergic neurons.

In the present study, choline acetyltransferase activity was decreased in the cortex by amyloid-β-(25–35) treatment, but not in the basal forebrain. The region of measurement as the basal forebrain was the region directly ventral to the anterior commisure containing the nucleus basalis, which is distant (about 2.5 mm) from the nearest lateral ventricle. The region of measurement as the cortex was the motor area of the frontoparietal cortex, which was located immediately above the lateral ventricle. Therefore, it is possible that amyloid-β-(25–35) might not diffuse from the lateral ventricle to all the region of the measured basal forebrain. In support of this possibility, the reduction of choline acetyltransferase-immunoreactive cells in the medial septum of amyloid-β-injected side was much greater than that of the contralateral side (data not shown), indicating that the diffusion of amyloid-β-(25–35) is limited.

Itoh et al. (1996) reported that high potassium- and nicotine-induced increase in acetylcholine release in the hippocampus and cerebral cortex was markedly impaired by continuous amyloid-β-(1–40) infusion by means of microdialysis, although the basal levels of acetylcholine in the amyloid-β-(1–40)-infused rats were not different from those in vehicle-infused control rats. Abe et al. (1994) similarly demonstrated that injection of amyloid-β-(12–28), amyloid-β-(25–35) or amyloid-β-(1–40) to the septum of rats resulted in a marked decrease in basal and potassium-evoked acetylcholine release. Moreover, injection of amyloid-β-(25–35) into the nucleus basalis decreased the basal acetylcholine release from the parietal cortex ipsilateral to the lesion (Giovannelli et al., 1995). In vitro experiments showed that amyloid-β-(1–28), amyloid-β-(25–35), amyloid-β-(1–40) and amyloid-β-(1–42) inhibited potassium-evoked acetylcholine release in the rat hippocampal and cortical slices (Kar et al., 1996). Amyloid-β-(1–28), amyloid-β-(25–35) and amyloid-β-(1–42) caused a reduction of intracellular concentration of acetylcholine as well as choline acetyltransferase activity in a cell line that expresses cholinergic characteristics (Pedersen et al., 1996). These results altogether suggest that amyloid-β induces the dysfunction of cholinergic neuronal systems. It is well known that a disruption of learning and cognitive function is associated with the central cholinergic hypofunction induced by scopolamine treatment (Molchan et al., 1992), by the lesioning of nucleus basalis and medial septum (Miyamoto et al., 1987) or during aging (Bartus et al., 1982).

In summary, we demonstrated that a single i.c.v. injection of amyloid-β-(25–35) induced learning deficits in passive avoidance and radial-arm maze tasks and reduced choline acetyltransferase activity in the medial septum, cortex and hippocampus. The decrease in the number of choline acetyltransferase-immunoreactive cells in the medial septum was also evident. These results led us to conclude that the dysfunction of cholinergic neuronal system induced by amyloid-β-(25–35) is associated with the disturbance of learning and cognitive function.

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References

Abe, E., Casamenti, F., Giovannelli, L., Scali, C., Pepeu, G., 1994. Administration of amyloid β-peptides into the medial septum of rats decreases acetylcholine release from hippocampus in vivo. Brain Res. 636, 162–164.

Albert, D.J., Chew, G.L., 1980. The septal forebrain and the inhibitory modulation of attack and defense in the rat. A review. Behav. Neural Biol. 30, 357–388.

Bartus, R.T., Dean III, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–417.

Bierer, L.M., Haroutunian, V., Gabriel, S., Knott, P.J., Carlin, L.S.,

Purohit, D.P., Perl, D.P., Schmeidler, J., Kanof, P., Davis, K.L., 1995. Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. J. Neurochem. 64, 749–760.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248–254.

Chen, S.-Y., Harding, J.W., Barnes, C.D., 1996. Neuropathology of synthetic β-amyloid peptide analogs in vivo. Brain Res. 715, 44–50.

Delobette, S., Privat, A., Maurice, T., 1997. In vitro aggregation facilitates β-amyloid peptide-(25–35)-induced amnesia in the rat. Eur. J. Pharmacol. 319, 1–4.

Dutar, P., Bassant, M.-H., Senut, M.-C., Lamour, Y., 1995. The septo-hippocampal pathway: structure and function of a central cholinergic system. Physiol. Rev. 75, 393–427.

Fonnum, F., 1975. A rapid radiochemical method for the determination of choline acetyltransferase. J. Neurochem. 24, 407–409.

Giovannelli, L., Casamenti, F., Scali, C., Bartolini, L., Pepeu, G., 1995. Differential effects of amyloid peptides β-(1–40) and β-(25–35) injections into the rat nucleus basalis. Neuroscience 66, 781–792.

Glenner, G.G., Wong, C.W., 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 120, 885–890.

Gray, J.A., McNaughton, N., 1983. Comparison between the behavioural effects of septal and hippocampal lesions: a review. Neurosci. Behav. Rev. 7, 119–188.

Harkany, T., O'Mahony, S., Kelly, J.P., Soos, K., Toro, I., Penke, B., Luiten, P.G.M., Nyakas, C., Gulya, K., Leonard, B.E., 1998. β-Amyloid(Phe$(SO_3H)^{24}$)25–35 in rat nucleus basalis induces behavioral dysfunctions, impairs learning and memory and disrupts cortical cholinergic innervation. Behav. Brain Res. 90, 133–145.

Harkany, T., Mulder, J., Sasvari, M., Abraham, I., Konya, C., Zarandi, M., Penke, B., Luiten, P.G.M., Nyakas, C., 1999. *N*-methyl-D-aspartate receptor antagonist MK-801 and radical scavengers protect cholinergic nucleus basalis neurons against β-amyloid neurotoxicity. Neurobiol. Dis. 6, 109–121.

Hoshi, M., Takashima, A., Murayama, M., Yasutake, K., Yoshida, N., Ishiguro, K., Hoshino, T., Imahori, K., 1997. Nontoxic amyloid β peptide 1–42 suppresses acetylcholine synthesis. J. Biol. Chem. 272, 2038–2041.

Itoh, A., Nitta, A., Nadai, M., Nishimura, K., Hirose, M., Hasegawa, T., Nabeshima, T., 1996. Dysfunction of cholinergic and dopaminergic neuronal systems in β-amyloid protein-infused rats. J. Neurochem. 66, 1113–1117.

Kar, S., Seto, D., Gaudreau, P., Quirion, R., 1996. β-amyloid-related peptides inhibits potassium-evoked acetylcholine release from rat hippocampal slices. J. Neurosci. 16, 1034–1040.

Kar, S., Issa, A.M., Seto, D., Auld, D.S., Collier, B., Quirion, R., 1998. Amyloid β-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. J. Neurochem. 70, 2179–2187.

Koshimura, K., Kato, T., Tohyama, I., Nakamura, S., Kameyama, M., 1986. Qualitative abnormalities of choline acetyltransferase in Alzheimer type dementia. J. Neurol. Sci. 76, 143–150.

Lin, L., Georgievska, B., Mattsson, A., Isacson, O., 1999. Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapse loss and muscarinic receptor activation. Proc. Natl. Acad. Sci. U. S. A. 96, 12108–12113.

Masters, C.L., Simms, G., Weinman, N.A., Malthaup, G., McDonald, B.L., Beyreuther, K., 1985. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc. Natl. Acad. Sci. U. S. A. 82, 4245–4249.

Maurice, T., Lockhart, B.P., Privat, A., 1996. Amnesia induced in mice by centrally administered β-amyloid peptides involves cholinergic dysfunction. Brain Res. 706, 181–193.

Miyamoto, M., Kato, J., Narumi, S., Nagaoka, A., 1987. Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Res. 419, 19–31.

Molchan, S.E., Martinez, R.A., Hill, J.L., Weingartner, H.J., Thompson, K., Vitiello, B., Sunderland, T., 1992. Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. Brain Res. Rev. 17, 215–226.

Nitta, A., Itoh, A., Hasegawa, T., Nabeshima, T., 1994. β-Amyloid protein-induced Alzheimer's disease animal model. Neurosci. Lett. 170, 63–66.

Olton, D.S., Samuelson, R.J., 1976. Remembrance of places passed: spatial memory in rats. J. Exp. Psychol.: Anim. Behav. Processes 2, 97–116.

Pavia, J., Alberch, J., Alverez, I., Toledano, A., de Ceballos, M.L., 2000. Repeated intracerebroventricular administration of β-amyloid 25–35 to rats decreases muscarinic receptors in cerebral cortex. Neurosci. Lett. 278, 69–72.

Paxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pedersen, W.A., Kloczewiak, M.A., Blusztajn, J.K., 1996. Amyloid β-protein reduces acetylcholine synthesis in a cell line derived from cholinergic neurons of the basal forebrain. Proc. Natl. Acad. Sci. U. S. A. 93, 8068–8071.

Pike, C.J., Burdick, D., Walencewicz, A.J., Glabe, C.G., Cotman, C.W., 1993. Neurodegeneration induced by β-amylod peptides in vitro: the role of peptide assembly state. J. Neurosci. 13, 1676–1687.

Pike, C.J., Walencewicz-Wasserman, A.J., Kosmoski, J., Cribbs, D.H., Glabe, C.G., Cotman, C.W., 1995. Structure–activity analyses of β-amyloid peptides: contributions of the β25–35 region to aggregation and neurotoxicity. J. Neurochem. 64, 253–265.

Selkoe, D.J., 1991. The molecular pathology of Alzheimer's disease. Neuron 6, 487–498.

Sigurdsson, E.M., Lee, J.M., Dong, X.-W., Hejna, M.J., Lorens, S.A., 1997. Bilateral injections of amyloid-β 25–35 into the amygdala of young Fischer rats: behavioral, neurochemical, and time dependent histopathological effects. Neurobiol. Aging 18, 591–608.

Sweeney, W.A., Luedtke, J., McDonald, M.P., Overmier, J.B., 1997. Intrahippocampal injections of exogenous β-amyloid induce postdelay errors in an eight-arm radial maze. Neurobiol. Learn. Mem. 68, 97–101.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., DeLong, M.R., 1982. Alzheimer's disease and senile dementia; Loss of neurons in the basal forebrain. Science 215, 1237–1239.

Yamaguchi, Y., Kobayashi, H., 1994. Effects of vasoactive intestinal peptide (VIP) on scopolamine-induced amnesia in the rat. Neuropeptides 26, 153–158.