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Research report

Implanted cannula-mediated repetitive administration of Aβ25–35 into the mouse cerebral ventricle effectively impairs spatial working memory

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Abstract

Amyloid β (Aβ) is closely related to the onset of Alzheimer's disease (AD). To construct AD animal models, a bolus administration of a large dose of toxic Aβ into the cerebral ventricles of rodents has been performed in earlier studies. In parallel, a continuous infusion system via an osmotic pump into the cerebral ventricle has been developed to make a rat AD model. In this study, we developed a mouse AD model by repetitive administration of Aβ25–35 via a cannula implanted into the cerebral ventricle. Using this administration system, we reproducibly constructed a mouse with impaired spatial working memory. In accordance with the occurrence of the abnormal mouse behavior, we found that the number of choline acetyltransferase (ChAT)-positive neurons was reduced in paraventricular regions of brains of Aβ25–35-administered mice in a dose-dependent manner. Considering that the repetitive administration of a small dose of toxic Aβ via an implanted cannula leads to a brain status more resembling that of the AD patients than a bolus injection of a large dose of Aβ, and therapeutic as well as toxic agents are able to be repeatedly and reliably administered via an implanted cannula, we concluded that the implanted cannula-bearing AD mouse model is useful for development of new AD therapy.

Keywords: Alzheimer's disease; Amyloid beta (Aβ); Implanted cannula; Y-maze test; Spontaneous alternation behavior

1. Introduction

Alzheimer's disease (AD) is characterized by progressive neuronal loss, intraneuronal tangles, and extracellular neuritic plaques. The major constituent of the plaque is amyloid β (Aβ), derived from amyloid β precursor protein (AβPP) [1]. Formation and accumulation of Aβ has been implicated in the development of AD [2–7]. Three genes with a missense mutation have been identified to cause familial AD (FAD): AβPP, presenilin 1 (PS1) and PS2 [8]. Multiple groups have found that constitutive overexpression of a FAD mutant increases production of toxic Aβ1–42/Aβ1–43 [9–13]. We have also reported that introduction of the London-type missense mutation (Val 642 Ile by the APP695 numbering) into the APP gene by a knock-in technique induced moderate cognitive impairment with increased ratio of Aβ42 (43)/Aβ40 [14]. Most importantly, it has been demonstrated that administration of anti-Aβ antibody attenuates not only the Aβ pathology but also the memory impairment of AD-model mice [15–18]. All these findings support the idea that Aβs are closely linked to the pathogenesis of AD.

Several AD-model mice have been developed by constitutively overexpressing FAD mutant genes using the transgenic mouse procedure [9–13,19]. Although massive neuronal loss does not occur in brains of these animals as it does in brains of human AD patients, the formation of plaque in brains as well as mild dementia occurs as they age. Consequently,

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these animals have been used as AD models with incomplete manifestations. In addition to these animal models, Flood et al. [20] first reported that intracerebroventricular (ICV) injection of Aβ peptides induced cognitive impairment in mice. Since then, multiple groups have reported that ICV injection of several kinds of Aβ peptides such as Aβ25–35, Aβ1–42, and Aβ1–40 induces memory impairment in mice and rats [21–28]. Because such Aβ model animals can be generated by shorter term procedures than transgenic mouse models, they have also been widely used as AD animal models. Administration of Aβ into cerebral ventricles has been usually performed by a one-shot or bolus ICV injection of a large dose of Aβ in these studies [21,25–28]. However, based on the fact that the concentrations of Aβ are kept upregulated without major fluctuation in AD brains for a long time, chronic Aβ neurotoxicity is considered to be more relevant in AD pathogenesis. Accordingly, it is preferable that Aβ peptides are continuously administered into the cerebral ventricle for a longer period. In this regard, although rats with dementia have been successfully constructed by continuous infusion of Aβ via an osmotic pump connected to the cerebral ventricle [22–24,29], until now an AD mouse model had not been developed using similar procedures.

In this study, we have developed an AD mouse model using a ventricle-implanted cannula system. Although this procedure is still not ideal from a standpoint of constantly upregulated Aβ concentration, the fluctuation of Aβ concentration by this method is considered to be much smaller than that by a bolus injection of a large dose of Aβ. This system enables us not only to observe more chronic effect of soluble toxic Aβ peptides in mice by repetitively delivering smaller doses of Aβ peptides directly into cerebral ventricles, but also to simultaneously administer therapeutic agents into the ventricle.

2. Materials and methods

2.1. Animal husbandry

This study was conducted in accordance with the Policies on the Use of Animals and Humans in Neuroscience Research, the Society for Neuroscience and Guideline for the Care and Use of Laboratory Animals of KEIO University School of Medicine. All the experimental procedures were approved by the Institutional Animal Experiment Committee at KEIO University. Animals were housed in a specific pathogen-free animal facility (23 ± 1 °C, 50 ± 5% humidity) under a 12-h light/dark cycle (7:00 a.m.–7:00 p.m.). They were fed ad libitum with gamma-ray irradiated Picolab Rodent Diet 20 (PMI Feeds Inc., St. Louis, MO) and sterile deionized distilled water (ddw) supplemented with sodium hypochlorite (5 ppm). Plastic cages (CLEA Japan, Tokyo, Japan) were autoclaved and ALPHA-dri bedding material (Shepherd Specialty Papers, Kalamazoo, MI) was sterilized by heating at 80 °C for 12 h before use and changed once a week. The mice had free access to food and water during task performance.

2.2. Implantation of cannulas and intracerebroventricular administration of Aβ

CD-1 mice at the age of 8 weeks weighing 33–37 g (Charles River Japan, Japan) were anesthetized with intraperitoneal injection of 10% Nembutal (sodium pentobarbital, 60 mg/kg) and placed in a stereotaxic surgery apparatus as described previously [30]. C315GS-4 cannula system for mice (Plastic One Inc., Roanoke, VA) was implanted into the left lateral ventricle under aseptic conditions through a hole drilled in the skull at the following coordinates: anterior–posterior, +0.3 mm; lateral, 1.0 mm; horizontal, 3.0 mm from the bregma, according to the atlas of Paxinos & Watson. The cannula was secured with surgical glue. Ten days after implantation, animals were randomly assigned to groups. C315IS-4 internal cannula (Plastic One Inc., Roanoke, VA) connected to a Hamilton syringe by cannula tubing (C232CT, PE50/Thin wall, Plastic One Inc., Roanoke, VA) was used for the injection.

We performed two experiments corresponding to Figs. 1 and 2. In the experiment in Fig. 1, mice were randomly assigned to two groups. One group received the bolus ICV injection, while the other received the repetitive ICV injection of Aβ peptide. For the bolus ICV injection, mice without cannula implantation were manually ICV injected with 5 μl of ddw or 10 nmol of Aβ25–35 in 5 μl of

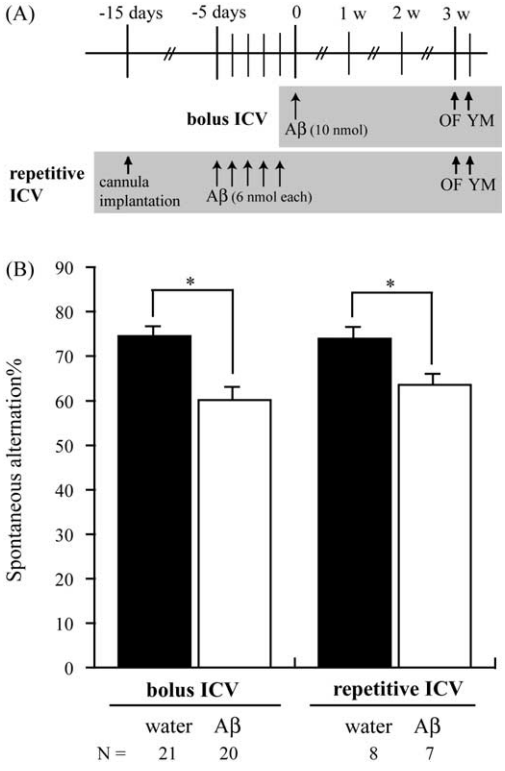

Fig. 1. Repeated intracerebroventricular administration of a smaller dose of Aβ25–35 via implanted cannula impairs Y-maze spontaneous alternation behavior. (A) An experimental schedule of Aβ25–35 administration. (B) SA% of mice singly injected with Aβ25–35 or repeatedly injected with a smaller dose of Aβ25–35 via implanted-cannula was significantly lower than that of water-injected control mice ($^{*}p < 0.05$). Data are shown in mean ± S.E.M. Statistical analyses were carried out by the one-way ANOVA followed by the Bonferroni–Dunn post hoc test.

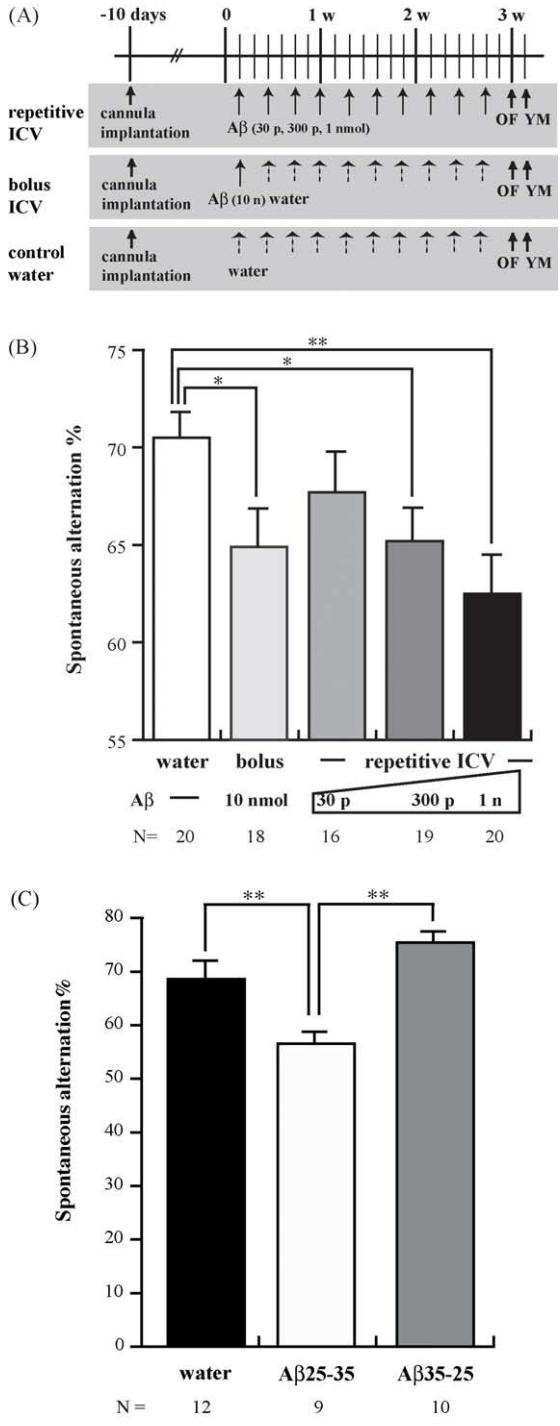

Fig. 2. Repetitive ICV administration of Aβ25–35 impairs Y-maze spontaneous alternation behavior in a dose-dependent fashion. (A) The experimental schedule of Aβ treatment. Mice were infused with 3 μl sterile ddw, stepwise increasing doses of Aβ25–35 (30 pmol, 300 pmol, or 1 nmol/2 days), or single 10 nmol Aβ25–35 plus repetitive 3 μl sterile ddw injection every other day for 3 weeks (10 times) and tested in OF and YM. (B) SA% of mice repetitively infused with Aβ25–35 at indicated doses/2 days and mice singly infused with 10 nmol Aβ25–35 was compared with that of water-injected control mice. (C) SA% of mice repetitively infused with Aβ25–35 or Aβ35–25 at 1 nmol/2 days was compared. Data are shown in mean ± S.E.M. Statistical analyses were carried out by one-way ANOVA followed by Bonferroni–Dunn post hoc test ($^{*}p < 0.05$, $^{**}p < 0.01$).

ddw (2 mM), as described in our ealier study [31]. For the repetitive ICV injection, mice were daily infused with 3 μl of ddw or 6 nmol of Aβ25–35 in 3 μl of ddw (2 mM) for 5 consecutive days corresponding to the 10th–14th days after the cannula was implanted. Twenty-one and 22 days after the last administration was performed, all mice were tested with the open field test (OF) and the Y-maze test (YM). In the experiment in Fig. 2, mice were infused with 3 μl of ddw or indicated amounts of Aβ25–35 or Aβ35–25 in 3 μl ddw every other day for 3 weeks (10 times). As a control bolus ICV injection, mice were infused with 10 nmol of Aβ25–35 in 5 μl of ddw (2 mM) on the 1st day, then the mice received an additional nine times injection of 3 μl ddw every other day for 3 weeks. Mice were tested with OF and YM 2 and 3 days after the last injection was performed.

2.3. Preparation of Aβ peptide

Aβ25–35 and Aβ35–25 were obtained from Peptide Institute (Osaka, Japan) and dissolved in sterile ddw. Various amounts of soluble Aβ25–35 (0, 30 pmol, 300 pmol and 1 nmol/2 days) without ageing procedure were repetitively infused for 3 weeks (10 times) via the implanted cannula.

2.4. Behavioral analyses

The open field test was carried out 3 weeks after single Aβ25–35 ICV injection or 2 days after ICV infusion repeated 10 times. The YM was also carried out on the next day after OF was carried out. All tests were performed by experienced researchers engaged in daily husbandry of the tested animals, in a quiet environment adjoining the room for permanent housing, after weaning. Experiments were carried out during the light phase, in a diffusely illuminated environment (400–500 lx). Following each trial, the surfaces of the testing apparatus were cleaned and deodorized with 70% alcohol and dried completely.

2.5. Open field test (OF)

OF was carried out as described previously [14]. Mice were individually placed at the center of a 100 cm^2 gray-plastic field (with 20-cm interval black grids) surrounded by a 20-cm wall, and allowed to move spontaneously for 3 min. Examined parameters were: (1) starting latency (defined as the time until the first grid line cross), (2) cumulative time spent in the peripheral area (within 20 cm of the wall), (3) total grid line crossing, and (4) activities such as rearing, defecation, grooming, urination, and preening (referred to as 'Events' in the following text).

2.6. Y-maze test (YM)

YM was carried out as described previously [31]. The maze was made of gray plastic, with each arm 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top. The three arms were connected at an angle of 120°. Mice were individually placed at the end of an arm and allowed to explore arms freely for 8 min. Examined parameters were: (1) frequency of entry into each arm, (2) total arm entries, (3) spontaneous alternation, and (4) 'Events'. Spontaneous alternation percentage (SA%) was defined as a ratio of the arm choices that differed from the previous two choices ("successful choices") to total choices during the run ("total entry minus two"

because the first two entries could not be evaluated). For example, if a mouse made 10 entries, such as 1-2-3-2-3-1-2-3-2-1, there are 5 successful choices in 8 total choices (10 entries minus 2). Therefore, SA% in this case is 62.5%.

2.7. Immunohistochemistry

Immunohistochemical analysis was performed as described previously [14,31]. After the behavioral experiments, mice were anesthetized, and transcardially perfused with phosphate buffered saline (PBS), and fixed by ethanol containing 5% acetic acid. Brains were embedded in paraffin, and 10-μm coronal sections were prepared on New-Silane slide glasses (Muto Pure Chemicals, Tokyo, Japan). Samples were subsequently dewaxed in xylene, and for immunohistochemical staining, they were washed sequentially in 100%, 90%, 70% ethanol, and finally, by water or phosphate-buffered saline. Immunohistochemical detection of cholinergic neurons was performed with anti-choline acetyltransferase (ChAT) antibody (1:50 dilution, Chemicon, USA) and visualized with ABC method (Vectastain Elite Kit, Vector, CA, USA). ChAT-immunoreactive neurons in the medial septum of five 10-μm thickness coronal sections with 50-μm interval [around 0.6–0.9 mm anterior from the bregma] were counted, and the averages of the total number of ChAT-immunoreactive neurons in three mice per treatment group ($N = 3$) were compared.

2.8. Immunoblot

Immunoblot analysis was performed, as described previously [30,32]. Cortical and hippocampal samples were separated from brains perfused with PBS, which were mechanically homogenized in a lysis buffer (50 mM Tris/HCl pH 7.4, 15 mM NaCl, 20 mM EDTA, 1% Triton X-100, Complete protease inhibitor). Lysate (50 μg/lane) from these parts was then subjected to SDS-PAGE and was electrically blotted to a polyvinylidene difluoride sheet. After blocking, the sheet was soaked with primary antibodies: anti-ChAT (1:250 dilution, Chemicon, USA). Immunoreactive bands were visualized by ECL (Amersham Pharmacia Biotech, Uppsala, Sweden).

2.9. Statistical analysis

All numerical data were tabulated by Microsoft Excel 2001 and statistically analyzed by StatView 5.0.1 software (SAS institute Inc., Cary, NC). Methods used for each analysis are indicated in each figure legend. Data are shown as mean ± S.E.M. The alpha value was set at $p < 0.05$.

3. Results

3.1. Repeated infusion of Aβ25–35 via an implanted cannula system is effective

We initially tried to confirm that a bolus injection of Aβ25–35 induced short spatial memory in mice, as described in earlier studies [21,25–28,31]. To this end, mice were injected with 5 μl of sterile deionized distilled water (ddw) or 5 μl of 2 mM Aβ25–35 (10 nmol totally) into cerebral ventricles, and then tested in OF and the YM after a 3-week interval, as shown in Fig. 1A. OF revealed that there is no statistically significant difference in total grid-line crossings, time spent in the peripheral area, and 'Events' between ddw- and Aβ25–35-injected mice, indicating that a bolus ICV injection of Aβ25–35 did not affect general behavior and motor function (Table 1). In contrast, YM showed that spontaneous alternation percentage of the control ddw-injected mice was 74.5% while that of Aβ25–35-injected mice was 60.2% (Fig. 1B, left), indicating that a bolus ICV injection of 10 nmol Aβ25–35 decreased SA% in YM as previously reported [31]. We confirmed that there was no statistically significant difference in total arm entries, the index of locomotor activities, between both groups (data not shown).

We then tested the efficacy and the side effect of repetitive delivery of a smaller dose of Aβ25–35 via an implanted cannula. We implanted a cannula into the left lateral ventricle of a mouse and daily infused 3 μl of ddw or 2 mM Aβ25–35 in 3 μl of ddw for 5 consecutive days (6 nmol/day) by inserting an internal cannula connected to a Hamilton syringe. Aβ25–35-injected mice were then tested with OF and YM after a 3-week interval (Fig. 1A). OF revealed no statistically significant difference in total grid line crossings, time spent in the peripheral area, and 'Events' between ddw- and Aβ-injected mice (Table 1). YM showed that SA% of ddw-injected mice was 73.9% (Fig. 1B, right), which is almost the same as singly ddw-injected mice or non-treated mice, indicating that implanted cannula-mediated repetitive infusion of 3 μl of ddw by itself did not affect SA%. On the other hand, SA% of repetitively Aβ25–35-delivered mice was reduced to 63.5%, indicating that repetitive ICV infusion of a smaller dose of Aβ25–35 caused substantial cognitive impairment (Fig. 1B, right, versus control, $p < 0.05$).

Table 1
Effect of single or repetitive intracerebroventricular administration of Aβ25–35 on general behavior in open field test (OF)

	N	Defecation	Urination	Grooming	Preening	Crossing	Peripheral	Latency	Rearing
Bolus ICV									
Water	21	1.00 ± 0.32	0.23 ± 0.19	0.04 ± 0.04	0.47 ± 0.11	115.7 ± 6.5	149.2 ± 2.9	5.1 ± 0.9	23.5 ± 1.8
Aβ	20	1.10 ± 0.33	0.45 ± 0.14	0.00 ± 0.00	0.65 ± 0.13	123.2 ± 5.9	141.7 ± 4.1	4.9 ± 0.6	20.2 ± 1.4
Repetitive ICV									
Water	8	1.25 ± 0.61	0.13 ± 0.13	0.00 ± 0.00	0.88 ± 0.23	116.9 ± 9.9	140.5 ± 8.9	6.0 ± 1.5	23.1 ± 4.9
Aβ	7	0.43 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	1.14 ± 0.14	122.7 ± 6.6	153.7 ± 4.3	4.5 ± 1.5	29.4 ± 1.2

Each value represents the mean ± S.E.M. Crossing: total grid line crossing; peripheral: total time spent in pheripheral area (s); latency: the time until the first grid line cross (s).

Table 2
Effect of repetitive intracerebroventricular administration of Aβ25–35 on general behavior in open field test (OF)

	N	Defecation	Urination	Grooming	Preening	Crossing	Peripheral	Latency	Rearing
Control	20	0.95 ± 0.34	0.30 ± 0.16	0.00 ± 0.00	0.25 ± 0.12	110 ± 7.9	146 ± 4.8	7.1 ± 1.4	19.4 ± 2.8
Bolus ICV	18	1.22 ± 0.51	0.50 ± 0.19	0.00 ± 0.00	0.33 ± 0.11	108 ± 5.0	146 ± 7.0	6.6 ± 1.1	16.5 ± 2.1
30 pmol	16	1.00 ± 0.41	0.38 ± 0.18	0.00 ± 0.00	0.31 ± 0.12	109 ± 6.8	150 ± 5.4	5.6 ± 1.3	24.3 ± 3.6
300 pmol	19	0.94 ± 0.43	0.58 ± 0.28	0.00 ± 0.00	0.53 ± 0.16	117 ± 8.5	155 ± 5.5	6.5 ± 1.5	21.9 ± 2.4
1 nmol	20	0.80 ± 0.30	0.40 ± 0.18	0.00 ± 0.00	0.50 ± 0.15	114 ± 5.3	152 ± 4.4	5.5 ± 0.9	24.3 ± 2.3

Each value represents the mean ± S.E.M. Crossing: total grid line crossing; peripheral: total time spent in pheripheral area (s); latency: the time until the first grid line cross (s).

3.2. Dose-dependent decrease in SA% by repetitive infusion of Aβ25–35 via the implanted cannula

In order to observe the chronic effect of toxic Aβ peptides in mice, we further modified the time schedule of the delivery of Aβ and behavior tests. We infused 3 μl of ddw or indicated amounts of Aβ25–35 in 3 μl ddw every other day for 3 weeks (10 times) and then tested mice with OF and YM (Fig. 2A). This protocol makes the condition of a mouse brain more resembling the in vivo condition of AD brains.

Using this protocol, we compared the effect of repetitive administration of stepwise increasing doses of Aβ25–35 using the implanted cannula system to optimize the delivered doses. Again, OF revealed no statistically significant difference in total grid-line crossings, time spent in the peripheral area, and 'Events' between ddw- and Aβ-injected mice in this protocol either (Table 2). However, YM showed that SA% of ddw-injected mice was 70.5% while repetitive infusion of smaller doses of Aβ25–35 induced memory impairment in a dose-dependent manner (Fig. 2B). We confirmed that there was no significant difference in total arm entries and 'Events' in YM (data not shown). Mean SA% of mice repetitively infused with Aβ25–35 at 1 nmol/2 days (total dose: 10 nmol/3 weeks) was significantly lower than the control (62.5%, $p < 0.01$). Similarly but less prominently, mean SA% of mice singly injected with 10 nmol Aβ25–35 was also significantly lower than the control (64.9%, $p < 0.05$). Altogether, we concluded that repetitive administration of small doses of Aβ25–35 induces memory impairment at least as efficiently as a bolus administration of a large dose of Aβ25–35 if the total injected doses are equal.

Finally, to exclude the possibility that the ICV injection of a large amount of short peptides itself non-specifically induces neurotoxicity, we compared the effect of Aβ25–35 with Aβ35–25, the control peptide with the reverse amino acid arrangement of Aβ25–35, in induction of cognitive impairment in YM. As shown in Fig. 2C, repetitive administration of Aβ25–35 reduced SA% in YM while that of Aβ35–25 did not, excluding this possibility.

3.3. Efficient loss of choline acetyltransferase (ChAT)-immunoreactive neurons by repetitive infusion of a small dose of Aβ25–35

We performed immunohistochemical and immunoblot analysis to examine whether repetitive infusion of small doses of Aβ25–35 induced downregulation of ChAT expression in neurons in paraventricular brain areas. We consequently found that the numbers of ChAT-immunoreactive neurons were reduced in the medial septum in a dose-dependent manner (Fig. 3A and B). Importantly, repetitive infusion of a small dose of Aβ25–35 more effectively reduced the number of ChAT-positive neurons than a bolus injection of a large dose of Aβ25–35. In agreement, immunoblot analysis indicated that expression of the ChAT-immunoreactive protein was downregulated in hippocampuses, but not in cortexes, in Aβ25–35-infused mice (Fig. 3C). These findings suggested that repetitive infusion of small doses of Aβ25–35 as well as a bolus injection of a large dose of Aβ25–35 induced memory impairment by reducing the number or the activity of cholinergic neurons in the septo-hippocampal region, which is one of the hallmarks of AD and is considered to be the main cause of memory impairment, as shown in earlier reports [31,33–36].

4. Discussion

We here show that the cannula implantation into the ventricle of a mouse, a rodent much smaller than a rat, is a safe and reproducible method of constructing a more appropriate AD model by repetitively delivering Aβ peptides. The cannula implantation itself does not induce any abnormal behavior in mice. The probability of cannula-related infection is estimated to be less than 1%.

We also definitively demonstrate that the ChAT expression level is reduced by administration of Aβ25–35, indicating that the memory impairment is induced by Aβ peptide-mediated inhibition of the cholinergic system in the hippocampus. This finding is basically in agreement with the foregoing reports [37]. It has also been shown that the cholinergic neurons especially in the basal forebrain, which play a major role in the memory system, are disrupted in AD brains [33,34]. In agreement, Donepezil, a compound that increases acetylcholine levels by interfering with the acetylcholine esterase activity, is clinically used for treatment of AD [38]. Even in a transgenic AD animal model, downregulation of activity of the cholinergic system is considered to be induced with overload of soluble Aβ peptides [39,40].

Repetitive administration of a small dose of Aβ25–35 via the implanted cannula impaired spatial working memory

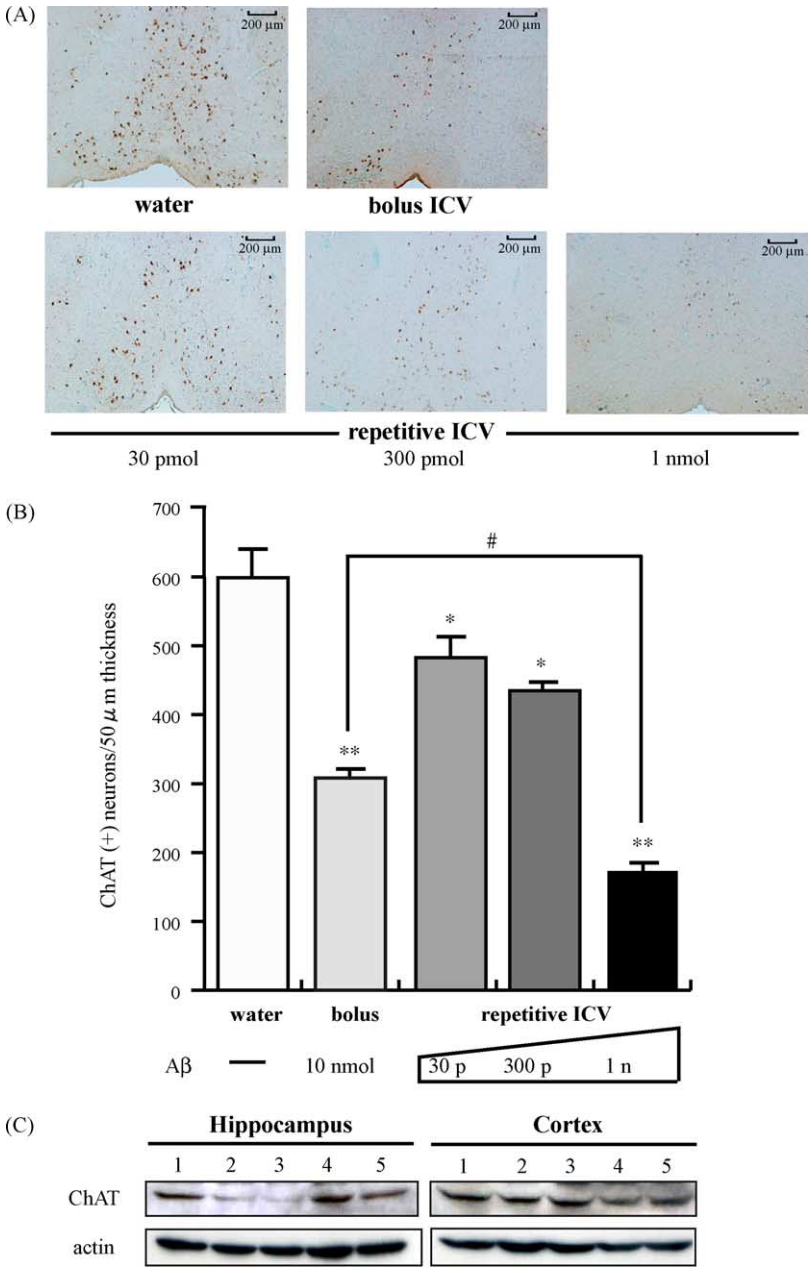

Fig. 3. Effect of Aβ25–35-repetitive injection on cholinergic neurons. (A) Coronal sections of the medial septum of mice repetitively infused with Aβ25–35 were examined by ChAT immunohistochemical staining. (B) The numbers of ChAT-immunoreactive neurons in the medial septum (10 μm thickness; 5 consecutive sections [total 50 μm thickness] located within 0.6–0.9 mm anterior from the bregma were counted) were compared ($N = 3$). Data are shown in mean ± S.E.M. Statistical analyses were carried out by one-way ANOVA followed by Fischer's PLSD. $^{*}p < 0.05$, $^{**}p < 0.01$ (vs. control [water]). There was a significant difference between bolus ICV (10 nmol) and repetitive ICV (1 nmol) ($^{\#}p < 0.05$). (C) Tissue lysates (50 μg/lane) of cortex and hippocampus were subjected to immunoblot analysis with anti-ChAT antibody and anti-actin antibody. 1: control (water injection); 2: bolus Aβ25–35 ICV (10 nmol); 3: repetitive Aβ25–35 ICV (1 nmol); 4: repetitive Aβ25–35 ICV (300 pmol); 5: repetitive Aβ25–35 ICV (30 pmol).

by reducing ChAT-positive neurons, as shown in Fig. 3. Because visualization of neurons with the Nissl staining and neuronal nuclei with the Hoechst33342 staining suggested that the number of neurons did not decrease and the number of apoptotic nuclei did not apparently increase (unpublished observation by M.Y., T.C., Y.K., and M.M.), we speculated that Aβ treatment downregulates the ChAT activity without death of ChAT-positive neurons. This has been already suggested in foregoing studies with ICV administration of Aβ peptides [29,37]. Accordingly, we may underestimate downregulation of the ChAT positivity by a bolus injection of Aβ, as compared with that by repetitive administration of Aβ because it is possible that the ChAT activity gradually returned to the normal levels for 21 days after a bolus injection of Aβ was performed. Therefore, we cannot simply conclude that repetitive administration of Aβ is superior

to a bolus injection of Aβ in downregulating the ChAT activity. Repetitive administration of Aβ, however, enables us to downregulate the ChAT activity constantly for longer periods. In this relation, we have already succeeded in administering a peptide via an implanted cannula for more than 2 months without any problematic complication [30].

Moreover, repetitive administration of a small dose of Aβ25–35 via the implanted cannula has several advantages, compared with one-shot ICV administration of a large dose of Aβ25–35. First, as shown in this study, the former procedure is at least as effective in inducing impairment of the spatial working memory. Second, implanted cannula-mediated repetitive administration appears to make a brain condition more similar to the in vivo brain condition of AD patients than a bolus injection of a large dose of Aβ peptides, because Aβ concentration in the brain is estimated to be moderately and constantly elevated for 3 weeks in this model. Third, because all AD animal models constructed with constitutive overexpression of FAD-related mutant genes are mice, findings obtained by our mouse AD model can easily be compared with accumulated findings obtained by transgenic mouse AD models.

In addition to these advantages, we could further find another advantage in this procedure. One of the major problems in mouse AD models constructed with single ICV administration of Aβ25–35 has been that it preferentially impairs short-term memory rather than long-term memory [28]. Considering that both short- and long-term memories are similarly impaired in AD patients and transgenic models [8,12], one of the reasons of preserved long-term memory in these mouse models constructed with the single ICV administration might be related to the lack of chronic Aβ neurotoxicity and rapid recovery of cognitive function including the cholinergic system. Until now, however, even using the repetitive-administration protocol shown in Fig. 2A, we have not been succeeded in constructing a mouse model with significantly impaired long-term memory (negative data not shown). We speculate on this issue that more chronic treatment with lower doses of Aβ would result in a mouse model with the impaired long-term memory. As already mentioned, we have succeeded in repetitive infusion of peptides for more than 2 months without any problematic complication enabling us to test longer procedures in the future investigation.

Finally, from the standpoint of drug discovery, the most prominent advantage of cannula implantation is that repeated administration of rescue factors as well as toxic factors is easily and faithfully accomplished without problematic stress to mice. Accordingly, this method is useful for testing efficacy of new therapeutic agents against AD-related disease models, especially when such new agents need to be directly administered into cerebrospinal fluids. Recently, we have succeeded in demonstrating the efficacy of derivatives of Humanin, a newly discovered factor protective against Alzheimer's disease's related neurotoxicity [32] against neurotoxicity by repetitive administration of a small dose of Aβ25–35 (unpublished observation by M.Y, T.C., J.S., Y.K., and M.M.). In addition, the procedure can be applied to other mouse neurodegenerative disease models. Using the cannula-implanted technique, we have succeeded in showing that repeated administration of ADNF, a neurotrophic factor, improves motor performance of amyotrophic lateral scrlerosis-model mice [30].

In conclusion, the cannula implantation system provides us with not only a more suitable toxic-Aβ-induced AD model but also an AD model by which efficacy of many therapeutic candidates can be tested more easily.

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References

[1] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987;325:733–6.
[2] Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, et al. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature 1992;360:672–4.
[3] Cairns NJ, Chadwick A, Lantos PL, Levy R, Rossor MN. βA4 protein deposition in familial Alzheimer's disease with the mutation in codon 717 of the βA4 amyloid precursor protein gene and sporadic Alzheimer's disease. Neurosci Lett 1993;149:137–40.
[4] Suzuki N, Cheung TT, Cai XD, Odaka A, Otvos Jr L, Eckman C, et al. An increased percentage of long amyloid beta protein secreted by familial amyloid β protein precursor (βAPP717) mutants. Science 1994;264:1336–40.
[5] Tomita T, Maruyama K, Saido TC, Kume H, Shinozaki K, Tokuhiro S, et al. The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid beta protein ending at the 42nd (or 43rd) residue. Proc Natl Acad Sci USA 1997;94:2025–30.
[6] Nevé RL, McPhie DL, Chen Y. Alzheimer's disease: a dysfunction of the amyloid precursor protein. Brain Res 2000;886:54–66.
[7] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353–6.

[8] Shastry BS, Giblin FJ. Genes and susceptible loci of Alzheimer's disease. Brain Res Bull 1999;48:121–7.
[9] Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1–42/1–40 ratio in vitro and in vivo. Neuron 1996;17:1005–13.
[10] Reaume AG, Howland DS, Trusko SP, Savage MJ, Lang DM, Greenberg BD, et al. Enhanced amyloidogenic processing of the beta-amyloid precursor protein in gene-targeted mice bearing the Swedish familial Alzheimer's disease mutations and a "humanized" Abeta sequence. J Biol Chem 1996;271:23380–8.
[11] Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G, et al. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. Nat Med 1997;3:67–72.
[12] Hock Jr BJ, Lamb BT. Transgenic mouse models of Alzheimer's disease. Trends Genet 2001;17:S7–12.
[13] Janus C, Phinney AL, Chishti MA, Westaway D. New developments in animal models of Alzheimer's disease. Curr Neurol Neurosci Rep 2001;1:451–7.
[14] Kawasumi M, Chiba T, Yamada M, Miyamae-Kaneko M, Matsuoka M, Nakahara J, et al. Targeted introduction of V642I mutation in amyloid precursor protein gene causes functional abnormality resembling early stage of Alzheimer's disease in aged mice. Eur J Neurosci 2004;19:2826–38.
[15] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173–7.
[16] Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000;408:982–5 [Erratum in Nature 2001;412:660].
[17] Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, et al. Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model. Nat Neurosci 2002;5:452–7.
[18] Gelinas DS, DaSilva K, Fenili D, St George-Hyslop P, McLaurin J. Immunotherapy for Alzheimer's disease. Proc Natl Acad Sci USA 2004;101(Suppl. 2):14657–62 [review].
[19] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, et al. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996;274:99–102.
[20] Flood JF, Morley JE, Roberts E. Amnestic effects in mice of four synthetic peptides homologous to amyloid beta protein from patients with Alzheimer disease. Proc Natl Acad Sci USA 1991;88:3363–6.
[21] Delobette S, Privat A, Maurice T. In vitro aggregation facilities β-amyloid peptide-(25–35)-induced amnesia in the rat. Eur J Pharmacol 1997;319:1–4.
[22] Yamada K, Tanaka T, Mamiya T, Shiotani T, Kameyama T, Nabeshima T. Improvement by nefiracetam of β-amyloid-(1–42)-induced learning and memory impairments in rats. Br J Pharmacol 1999;126:235–44.
[23] Yamada K, Tanaka T, Han D, Senzaki K, Kameyama T, Nabeshima T. Protective effects of idebenone and alpha-tocopherol on β-amyloid-(1–42)-induced learning and memory deficits in rats: implication of oxidative stress in β-amyloid-induced neurotoxicity in vivo. Eur J Neurosci 1999;11:83–90.
[24] Olariu A, Tran MH, Yamada K, Mizuno M, Hefco V, Nabeshima T. Memory deficits and increased emotionality induced by β-amyloid (25–35) are correlated with the reduced acetylcholine release and altered phorbol dibutyrate binding in the hippocampus. J Neural Transm 2001;108:1065–79.
[25] Yamaguchi Y, Kawashima S. Effects of amyloid-β-(25–35) on passive avoidance, radial-arm maze learning and choline acetyltransferase activity in the rat. Eur J Pharmacol 2001;412:265–72.
[26] Sun MK, Alkon DL. Impairment of hippocampal CA1 heterosynaptic transformation and spatial memory by β-amyloid25–35. J Neurophysiol 2002;87:2441–9.
[27] Mazzola C, Micale V, Drago F. Amnesia induced by β-amyloid fragments is counteracted by cannabinoid CB_1 receptor blockade. Eur J Pharmacol 2003;477:219–25.
[28] Stepanichev MY, Moiseeva YV, Lazareva NA, Onufriev MV, Gulyaeva NV. Single intracerebroventricular administration of amyloid-beta (25–35) peptide induces impairment in short-term rather than long-term memory in rats. Brain Res Bull 2003;61:197–205.
[29] Nitta A, Fukuta T, Hasegawa T, Nabeshima T. Continuous infusion of β-amyloid protein into the rat cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Jpn J Pharmacol 1997;73:51–7.
[30] Chiba T, Hashimoto Y, Tajima H, Yamada M, Kato R, Niikura T, et al. Neuroprotective effect of activity-dependent neurotrophic factor against toxicity from familial amyotrophic lateral sclerosis-linked mutant SOD1 in vitro and in vivo. J Neurosci Res 2004;15:542–52.
[31] Tajima H, Kawasumi M, Chiba T, Yamada M, Yamashita K, Nawa M, et al. A Humanin derivative, S14G-HN, prevents amyloid-β-induced memory impairment in mice. J Neurosci Res 2005;79:714–23.
[32] Hashimoto Y, Niikura T, Tajima H, Yasukawa T, Sudo H, Ito Y,et al. A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and Aβ. Proc Natl Acad Sci USA 2001;98:6336–41. [Erratum in Proc Natl Acad Sci USA 2001;98:12854].
[33] Bartus RT, Dean III RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982;217:408–17.
[34] Coyle JT, Price DL, DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 1983;216:1184–90.
[35] Stepanichev MY, Zdobnova IM, Zarubenko II, Moiseeva YV, Lazareva NA, Onufriev MV, et al. Amyloid-β(25–35)-induced memory impairments correlate with cell loss in rat hippocampus. Physiol Behav 2004;80:647–55.
[36] Yamaguchi Y, Matsuno T, Kawashima S. Antiamnesic effects of azaindolizinone derivative ZSET845 on impaired learning and decreased ChAT activity induced by amyloid-β 25–35 in the rat. Brain Res 2002;945:259–65.
[37] Tran MH, Yamada K, Nabeshima T. Amyloid-peptide induces cholinergic dysfunction and cognitive deficits: a minireview. Peptides 2002;23:1271–83 [review].
[38] Barner EL, Gray SL. Donepezil use in Alzheimer disease. Ann Pharmacother 1998;32:70–7.
[39] Luth HJ, Apelt J, Ihunwo AO, Arendt T, Schliebs R. Degeneration of beta-amyloid-associated cholinergic structures in transgenic APP SW mice. Brain Res 2003;977:16–22.
[40] Ohno M, Sametsky EA, Younkin LH, Oakley H, Younkin SG, Citron M, et al. BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron 2004;41:27–33.