Memory Impairment and Neuronal Dysfunction Induced by β -Amyloid Protein in Rats

TOSHITAKA NABESHIMA and ATSUMI NITTA

Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466

NABESHIMA, T. and NITTA, A. *Memory Impairment and Neuronal Dysfunction Induced by β -Amyloid Protein in Rats.* Tohoku J. Exp. Med., 1994, 174 (3), 241-249 — Alzheimer's disease (AD) is characterized by the presence of senile plaques. The core of the plaque consists of β -amyloid protein. In AD patients, learning and memory are impaired with a concomitant loss of the cholinergic marker enzyme, choline acetyltransferase (ChAT). However, direct evidence that β -amyloid protein is related to the impairment of learning and memory has not been demonstrated. In this study, we investigated whether memory impairment and neuronal dysfunction were produced after 2 weeks continuous infusion of β -amyloid protein (3, 30 and 300 pmol/day) into the cerebral ventricles in adult rats. To investigate the ability of learning and memory in β -amyloid protein-treated rats, water maze and passive avoidance tasks were carried out. The performance of both tasks in β -amyloid protein-treated rats was impaired. ChAT activity in the frontal cortex (3 and 30 pmol/day) and hippocampus (300 pmol/day) significantly decreased. These results suggest that β -amyloid protein is related to the impairment of learning and memory, and neurodegeneration, and that β -amyloid protein-treated rats could be used as an animal model for AD. ——— β -amyloid protein; Alzheimer's disease; memory; choline acetyltransferase; rats

Alzheimer's disease (AD) is characterized by the presence of senile plaques and neurofibrillary tangles accompanied by synaptic and neuronal loss. The core of the plaque consists of β -amyloid protein (Masters et al. 1985; Abraham et al. 1988) and other proteins (Show et al. 1988). The extent of β -amyloid protein deposition correlates with the degree of neuronal damage, cognitive impairment, and memory loss (Wilcock and Esiri 1982; Mann et al. 1985). Although this protein has been well characterized biochemically, its primary biological function and role in the pathogenesis of AD are unknown (Müller-Hill and Beyreuther

Received July 18, 1994; revision accepted for publication September 5, 1994.

Address for reprints: Toshitaka Nabeshima, Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Tsuruma-cho 65, Showaku, Nagoya 466, Japan.

1984).

Yankner et al. (1990), have reported that β -amyloid protein in vitro has both neurotrophic and neurotoxic effects that depend on neuronal age and the concentration of β -amyloid protein. Additionally, they have shown that fragments of β -amyloid, as well as the complete peptide sequence of β -amyloid (1–40), were neurotoxic to the hippocampus cells in primary culture and that direct injection of β -amyloid into the hippocampus of rats resulted in a pattern of characteristic changes of AD; β -amyloid induces Alz-50-immunoreactive protein in rat cerebral cortex that was very similar to the protein induced in human cerebral cortex from patients with AD (Kowall et al. 1991).

In AD patients, learning and memory are impaired by the concomitant loss of the cholinergic marker enzyme, choline acetyltransferase (ChAT), in the cerebral cortex (Wilcock et al. 1982). However, direct evidence, that β -amyloid protein is related to the impairment of learning and memory, has not been obtained. We now show that learning and memory impairment and neuronal dysfunction of cholinergic neurons were produced after continuous infusion of β -amyloid protein into the cerebral ventricles in adult rats. These results in this study, suggest that the deposition of β -amyloid protein in the brain is related to the impairment of learning and memory, and the cholinergic neuronal degeneration, and that β -amyloid protein-treated rats could be used as an animal model for AD.

MATERIALS AND METHODS

Animals

Male Kbl Wistar rats (Oriental Bioservice Co., Kyoto), weighing 280–320g at the beginning of the experiments, were used. They were housed in groups of two or three in a temperature- and light-controlled room (23°C; 12-hr light cycle starting at 9:00 a.m.). The rats had free access to food and water, except during the experiments.

Surgery and experimental design

The synthesized human β -amyloid protein (1–40) was kindly provided by Shionogi Co. Ltd. (Osaka). The β -amyloid protein was dissolved in 35% acetonitrile/0.1% trifluoroacetic acid (TFA). Continuous infusion of the β -amyloid protein (0, 3, 30, 300 pmol/day) was maintained for two weeks by attachment of a cannula to a modified miniosmotic pump (Alzet 2002; Alza, Palo Alto, CA, USA) (Nabeshima et al. 1991). Each group consisted of seven rats. The cannula was implanted into the left ventricle (A-0.3, L1.1, V3.6) on the day 1. The water maze task and passive avoidance task were carried out on day 9 to day 13 and on day 14 to day 15, respectively, after the start of infusion. After the behavioral experiments, four rats of each group were decapitated for ChAT and cholinesterase (ChE) activity assays.

Histochemical study

Three rats were used for histochemical study. In the histochemical study, rats were anesthetized and killed by transaortic perfusion-fixation with cold saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brains were removed, postfixed for 12 hr in the same fixative. The brains were left in 10% sucrose in phosphate-buffered saline (PBS) for 4 hr, 15% one for 4 hr, and 20% one for 12 hr at 4°C. Frozen brains were cut at 20 μ m with a cryostat and collected the areas around the ventricles. A mouse anti-human β -amyloid protein (8-17) monoclonal antibody (Dakopatts A/C; Glostrup, Denmark) was used at a 1 : 100 dilution. The tissues were incubated overnight at 4°C with the primary antibody. Immunolabeled sections were processed and developed with diaminobenzidine using Vectastain kit (Vector Laboratories, Burlingame, CA, USA).

Water maze task

Water maze task was carried out as reported previously (Morris 1984; Nitta et al. 1993b). The animal was trained for 90 sec. When it failed to get to the hidden platform, the training was terminated and a maximum score of 90 sec was assigned. Training was carried out twice a day. Training was conducted on 5 consecutive days (two trials x sessions of two trial each).

Step-through passive avoidance task

Step-through passive avoidance task was carried out as reported previously (Nitta et al. 1993a). The criterion was whether the animals remained in the light compartment for at least 300 sec. The results were expressed as the percent of animals per group that showed a step-through latency of 300 sec or more (Retention %).

Measurement of ChAT and ChE activity

Measuremen for ChAT and ChE activity was carried out as reported previously (Ellman et al. 1961; Kaneda and Nagatsu 1985; Nitta et al. 1993a).

Statistical analysis

The data from the maze task were analyzed by a repeated-measure analysis of variance and Tukey's test. ChAT activity data were analyzed using one-way analysis of variance and Tukey's test.

Fig. 1. The deposition of β -amyloid protein in the brain of rat demonstrated by immunohistochemical study.

RESULTS

The deposition of β -amyloid protein in the brain of rat demonstrated by immunohistochemical study

The immunohistochemical staining showed the accumulation of β -amyloid protein in the hippocampus and cerebral cortex 14 days after β -amyloid protein infusion (Fig. 1).

Characteristics of impairment of the performance in the water maze task induced by β -amyloid protein

In the water maze task, the mean values for the latencies of the 4 groups (to escape onto the hidden platform) in each training period of the water maze task are shown in Table 1. The latencies in the vehicle-treated group in the first training period were not different from those in the β -amyloid-treated rats. Although repeated trainings slowly shortened the latencies in the β -amyloid-treated (especially 300 pmol/day) groups, rapidly shortened those in the vehicle-treated group [$F(3, 9)=10.00$, $p<0.005$]. In the 6, 7, 8 and 10 training periods, the latencies of β -amyloid protein (300 pmol/day)-treated rats were longer than those of the vehicle-treated group (Tukey's test, $p<0.05$).

Characteristics of impairment of the performance in the passive avoidance task induced by β -amyloid protein

Each value in Fig. 2 represent the percent of animals which reached the criterion of 300 sec-step-through latency (retention %). The retention percent of

TABLE 1. *Effects of continuous infusion of β -amyloid protein on the performance in water maze task*

	Trial No.									
	1	2	3	4	5	6	7	8	9	10
β -amyloid protein(pmol/day)										
0	90 ± 0.0	82.8 ± 6.9	71.4 ± 12.3	50.6 ± 11.1	41.5 ± 12.1	31.6 ± 10.3	33.6 ± 10.6	31.9 ± 11.2	55.1 ± 15.0	33.7 ± 14.6
3	85.9 ± 4.1	90.0 ± 0.0	62.6 ± 12.4	74.6 ± 10.8	67.0 ± 9.6	53.4 ± 14.3	49.8 ± 12.9	61.4 ± 12.9	55.6 ± 14.3	43.6 ± 14.3
30	90.0 ± 0.0	90.0 ± 0.0	75.7 ± 12.5	76.1 ± 13.9	66.7 ± 15.2	55.1 ± 15.9	55.1 ± 16.5	48.7 ± 18.5	38.9 ± 16.6	35.8 ± 17.3
300	90.0 ± 0.0	82.3 ± 7.7	83.0 ± 7.0	78.5 ± 11.5	79.2 ± 10.8	83.0* ± 7.0	78.6* ± 11.4	78.4* ± 11.6	78.4 ± 7.1	67.6* ± 14.5

Each value shows the mean of the latency (sec) of each training. The task was carried out on 14 to day 15 after the start of infusion of β -amyloid protein.

* $p < 0.05$ vs. vehicle (β -amyloid protein: 0 pmol/day)-treated rats (Tukey's test).

Fig. 2. Effects of continuous infusion of β -amyloid protein on the performance in passive avoidance task. The task was carried out on day 14 to day 15 after the start of infusion of β -amyloid protein. Retention % is the percent of animals per group that showed a step-through latency of 300 sec or more.

the β -amyloid protein-treated rats was smaller than that of the vehicle-treated rats ($\chi^2 = 11.551$, $\alpha = 0.0091$).

TABLE 2. *Effects of continuous infusion of β -amyloid protein on ChAT and ChE activities in the frontal cortex, parietal cortex, striatum, and hippocampus in rats*

Brain region	β -Amyloid protein (pmol/day)			
	0	3	30	300
ChAT activity (nmol/min/g) immediately after cessation				
Frontal cortex	1196.4 $\pm$ 22.4	849.6* $\pm$ 28.6	833.7* $\pm$ 53.5	895.8 $\pm$ 43.4
Parietal cortex	718.9 $\pm$ 45.3	647.5 $\pm$ 45.4	659.0 $\pm$ 44.0	714.8 $\pm$ 67.6
Striatum	4041.7 $\pm$ 691.6	4309.7 $\pm$ 253.2	4244.8 $\pm$ 206.8	4318.0 $\pm$ 344.6
Hippocampus	899.7 $\pm$ 56.9	826.0 $\pm$ 67.8	814.7 $\pm$ 12.9	739.0* $\pm$ 45.7
ChE activity (μ mol/hr/mg)				
Frontal cortex	1018.9 $\pm$ 35.9	904.3 $\pm$ 36.4	933.8 $\pm$ 67.4	1035.2 $\pm$ 42.9
Parietal cortex	943.4 $\pm$ 52.6	940.1 $\pm$ 72.6	912.5 $\pm$ 35.1	1235.4 $\pm$ 272.0
Striatum	9353.9 $\pm$ 1199.9	1150.4 $\pm$ 94.2	1225.4 $\pm$ 144.4	10034.5 $\pm$ 59.3
Hippocampus	1350.0 $\pm$ 64.0	9614.0 $\pm$ 494.6	9907.2 $\pm$ 392.6	1258.2 $\pm$ 669.8

Each value shows the mean $\pm$ s.e. of four animals. Rat was decapitated and the brain was removed for ChAT and ChE activity assays after the two week continuous infusion.

* $p < 0.05$ vs. vehicle (β -amyloid protein: 0 pmol/day)-treated rats (Tukey's test).

Effects of continuous infusion of β -amyloid protein on ChAT and ChE activity in the frontal cortex, parietal cortex, striatum, and hippocampus in rats

ChAT activity in the frontal cortex, parietal cortex, striatum and hippocampus in the vehicle-treated rats was 1196.4 ± 22.4 , 718.9 ± 45.3 , 4041.7 ± 691.6 and 899.7 ± 56.9 nmol/min/g protein, respectively. ChAT activity in the frontal cortex (3, 30 pmol/day) and hippocampus (300 pmol/day) was decreased by β -amyloid protein infusion (Table 2).

ChE activities in the frontal cortex, parietal cortex, striatum and hippocampus in the vehicle-treated rats were 1018.9 ± 35.9 , 943.4 ± 52.6 , 9353.9 ± 1199.9 and 1350.0 ± 64.0 μ mol/hr/mg protein, respectively. There were no differences in ChE activity between the vehicle- and β -amyloid protein-treated rats in all brain regions (Table 2).

DISCUSSION

The important findings in this study are that the deposition of β -amyloid protein in the brain is related to disability of learning and memory, and the cholinergic neuronal degenerations. In the water maze and passive avoidance tasks, the performance of β -amyloid protein-treated rats was impaired with degeneration of the cholinergic neurons. Furthermore, we observed a delay of habituation to circumstances in β -amyloid protein-treated animals (data not

shown). A previous report has demonstrated that a single injection of fragment of β -amyloid protein (1-28) causes impairment of performance in footshock active avoidance task in mice (Flood et al. 1991). It is the first finding that the continuous infusion of β -amyloid protein into ventricles has amnesic effects in a spatial memory task. Our experimental conditions by using *continuous* infusion method of β -amyloid protein (1-40) are very similar to the deposition process of β -amyloid protein in AD patients. Further, the decrease of ChAT activity, one of the marker enzymes for cholinergic neuronal dysfunctions, was observed in the frontal cortex and hippocampus in β -amyloid protein-infused groups.

Attempts to demonstrate neurotoxic effects of β -amyloid protein and related peptides have met with mixed results. Early work by Whitson et al. (1989) has shown that a synthetic peptide corresponding first 28 amino acids of β -amyloid protein causes a dose-dependent trophic effect on the rat hippocampal neurons in vitro (Whitson et al. 1989). Using the full-length of β -amyloid protein in the same paradigm, it has been demonstrated neurite-promoting effects are characterized by both extensive dendritic branching and the neurotoxic effects of glutamate on mouse cortical neurons in vitro (Koh et al. 1990). Yankner et al. (1990) has reported that the early neurotrophic effects is observed but there is no neurotoxicity when β -amyloid protein is added to the hippocampal cultures at a low concentration. Furthermore, following incubation under the physiological conditions β -amyloid protein adopts an aggregated form and shows a change in its biological effects on the hippocampal neurons in vitro from neurite-promoting to neurotoxic effects (Pike et al. 1991). These results suggest that the aged peptide has an altered, aggregated structure evidenced by the stability of high molecular weight species and that the aggregated forms may related to the observed toxicity. In present study, the neuronal degeneration may be also related to the aggregated protein, since β -amyloid protein was incubated in the cerebrospinal fluid (CSF) for 14 days after the infusion. However, it is difficult to confirm this hypothesis, since mass spectroscopy and amino acid sequence analysis were essential to determine whether the incubated and non-incubated β -amyloid protein were the same structures or not (Pike et al. 1991).

We dissolved β -amyloid protein with acetonitrile/TFA in this study. From previous solubility studies of β -amyloid protein, solution of β -amyloid protein at 15 mg/ml (the concentration is 10 times higher than the highest dose in the present study) does not precipitate after 24 hr at 22 or 37°C in acetonitrile/TFA (Waite et al. 1992). When β -amyloid protein is dissolved in water or PBS, β -amyloid protein precipitates following incubation under the physiological conditions (Waite et al. 1992). If β -amyloid protein is precipitated in the osmotic pump, it can't be delivered from the pump to the brain. Acetonitrile toxicity in vivo has been reported to be linked to conversion to cyanide by microsomal cytochrome P450 (Freeman and Hayes 1988). Cyanide toxicity, like hypoxia, is known to involve a compromised calcium homeostasis caused by deficiency of energy

metabolism (Goldberg et al. 1987). It is also plausible to hypothesize that the 35% acetonitrile may extract membranes and allow toxic calcium influx. Calcium-mediated neurotoxicity has been shown to be potentiated by β -amyloid protein (Mattson et al. 1992). Acute injection of acetonitrile into the rat hippocampus gives very serious damage to the neuronal cells (Waite et al. 1992). However, neuronal damage was not observed under the present condition; the solvent containing β -amyloid protein was infused continuously (2 weeks) and slowly ($0.5 \mu\text{l/hr}$) with mini osmotic pump into the cerebral ventricles. In the preliminary experiments, there was no differences between the acetonitrile-treated and intact rats in the learning and memory and activity of ChAT. Based upon these observations, we used acetonitrile as the solvent for β -amyloid protein.

Now we are investigating whether our animal model for AD shows other characteristics of AD and whether this animal model is useful for an evaluation of cognitive enhancers.

In conclusion, the present results suggest that the deposition of β -amyloid protein in the brain is related to the impairment of learning and memory, and the cholinergic neuronal degeneration, and that β -amyloid protein-treated rats could be used as an animal model for AD.

Acknowledgments

This study was supported in part by grants from the Ministry of Health and Welfare Foundation for Gerontological Science Research (91A-2406), SRF Grant for Biomedical Research, Japan Research Foundation for Clinical Pharmacology and the Japan Brain Foundation.

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