INTRODUCTION

Senile plaque (SP), one of characteristic features of Alzheimer's disease (AD), mainly consists of \( \beta \)-amyloid peptide (A\( \beta \))\(^1\). In AD patients, cognitive functions are impaired with concomitant loss of the cholinergic neuron in the brain\(^2\). One hypothesis-accumulation of A\( \beta \) is responsible for neurodegeneration in AD-is led by the results that A\( \beta \) showed neurotoxicity in vitro\(^3,4\), however, the mechanisms underlying neurodegeneration are still unknown.

To investigate the in vivo toxicity of A\( \beta \), therefore, we infused A\( \beta \) into the cerebral ventricles of rats. The toxicity of A\( \beta \) infusion was evaluated by behavioral, neurochemical, electrophysiological and histological methods.

MATERIALS AND METHODS

A\( \beta \)-induced animal model

Male Wistar rats (280-320g) were infused synthesized human A\( \beta \) (1-40) dissolved in 35% acetonitrile/0.1% trifluoroacetic acid. Continuous infusion of A\( \beta \) (3, 30 and 300 pmol/day) was maintained by a modified osmotic minipump\(^5,8\). Control rats was infused with vehicle instead of A\( \beta \).

Behavioral evaluation

The ability of learning and memory was evaluated by Y-maze (day 7), habituation (day 8 and 9), Morris's water maze (day 9-13) and passive avoidance task (day 14 and 15). Details of procedures of behavioral tests were described previously\(^6,12\).

Neurochemical analysis

For the assay of choline acetyltransferase (ChAT) activity, rat brains were removed 16 days after A\( \beta \) infusion. Methods for ChAT assay was reported previously\(^12\).

In vivo brain microdialysis was carried out 10-12 days after the start of A\( \beta \) infusion (300 pmol/day). Acetylcholine (ACh) in the frontal cortex/hippocampus and dopamine (DA) in the striatum were detected by HPLC-ECD\(^3,14\).

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Fig. 1. Effects of continuous infusion of A\( \beta \) into the cerebral ventricle of rats on learning and memory (A) and on neurotransmitter release (B, C and D).

(A) The results was expressed mean escape latency in Morris's water maze.

B) F(1,92)=6.866, p<0.05 (2-way ANOVA); **p<0.01 vs. Control (Scheffé's test).

C) F(9,64)=2.267, p<0.05 (2-way ANOVA), D) F(1,79)=16.43, p<0.01 (2-way ANOVA); **p<0.01 vs. Control (Scheffé's F test).
Electrophysiological analysis

Ten or 11 days after Aβ (300 pmol/day) infusion, 400 μm-thick brain slices of the hippocampus were prepared for extracellular recordings. Electrical stimulation was applied to Schaffer collaterals and recording was performed in the CA1 pyramidal cell layer.

Immunohistochemical analysis

Rats were anesthetized and killed by transaortic perfusion-fixation 16 days after Aβ infusion, and then Aβ and glial fibrillary acidic protein (GFAP) were stained by immunoassay.

RESULTS

Impairment of learning and memory

In the Y-maze task, the percent of spontaneous alternation behavior was decreased by Aβ (300 pmol/day) treatment (data not shown). In the habituation task, the Aβ (300 pmol/day)-infused group showed more spontaneous locomotor activity compared to control. Thus, the habituation to the experienced environment in the Aβ-infused rats was poorer than that in control (data not shown).

At the 6, 7, 8, and 10th training periods of water maze task, Aβ-infused rats, particularly 300 pmol/day group, needed longer time to escape onto the hidden platform compared to control (Fig. 1A)5-8.

The results of the passive avoidance task are summarized in Table 1. At the retention trial, the number of animals which reached the criterion of 300 sec in the Aβ (300 pmol/day)-infused group was smaller than that in control5-8.

Dysfunction of cholinergic and dopaminergic neuronal systems

ChAT activity in the frontal cortex and hippocampus was shown in Table 1, and that in the frontal cortex (3, 30 pmol/day) and hippocampus (300 pmol/day) was significantly decreased by Aβ infusion5-8.

As shown in Fig. 1B and C, the perfusion of nicotine increased the extracellular levels of ACh and DA in the frontal cortex/hippocampus and striatum, respectively, of control. In the Aβ-infused group, however, the nicotine-stimulated releases of ACh and DA were significantly lower than these in control19-24. Further, a dramatic increase in the extracellular level of DA in the striatum of control was induced by high K-stimulation, however, that in the Aβ-infused group was significantly lower compare to control (Fig. 1D)19.

Deficiency of nicotinic response and LTP formation in the hippocampal CA1

Application of nicotine (50 μM, 10 min) reduced amplitude of population spike in the hippocampal CA1 of control, but not of Aβ-infused rats (data not shown). After the tetanic stimulation, enhanced responses were observed and it was maintained at about 2-fold larger responses compared to the basal level for more than 45 min in control, suggesting the induction of LTP (data not shown). However, the enhanced responses in the Aβ-infused group declined rapidly to near baseline.

Histological changes

The immunohistochemical staining showed the accumulation of Aβ in the hippocampus and cerebral cortex 14 days after Aβ infusion5-8. Vimentine and GFAP immunoreactivity increased both immediately and 2 weeks after the Aβ infusion in the hippocampus CA1 area5-8 (data not shown).

DISCUSSION

In the Aβ-infused rats, the ability of learning and

<table>
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<th>Table 1</th>
<th>Impairments of performance on passive avoidance task and of ChAT activity in the Aβ-infused rats</th>
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<tbody>
<tr>
<td>Control</td>
<td>7 (8)</td>
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<tr>
<td>Aβ (pmol/day)</td>
<td>3</td>
</tr>
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<td></td>
<td>30</td>
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The passive avoidance task was carried out 14-15 days and ChAT activity was determined 16 days after the start of Aβ infusion. No. of animals represents the numbers of rats that reached criterion (300 sec) and the numbers of rats used in passive avoidance task are noted in the parenthesis. *p<0.05, **p<0.01 vs. Control.
memory evaluated by habituation, Y-maze, Morris's water maze and passive avoidance tasks was impaired. Neurochemical study showed decrease of ChAT activity and release of neurotransmitters in Aβ-infused rats. Therefore, it can be considered that Aβ-induced neuronal dysfunction is responsible for impairment of learning and memory. In vivo brain microdialysis and in vitro electrophysiological studies also showed that Aβ may impair the signal transduction via nicotinic ACh receptors.

Moreover, the induction of LTP, an attractive candidate mechanism for memory storage, was suppressed in the Aβ-infused rats. Therefore, the impairment of learning and memory observed in the Aβ-infused rats may be based on the deficiency of LTP induction following impairment of neurotransmitter release. At present, we do not know the exact mechanism of Aβ-induced toxicity in the neuronal systems. However, we suggest that continuous infusion of Aβ impaired the neuronal activity by inducing a disruption of a physiological function such as Ca2+ homeostasis, as reported by Mattson's group.

The results of the present studies show that continuous infusion of Aβ impaired learning and memory and release of neurotransmitters, suggesting learning deficits can be interpreted as caused by impairment of neurotransmitter release.

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REFERENCES


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