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Preparation of Alzheimers Animal Model and Brain Dysfunction Induced by Continuous β-Amyloid Protein Infusion

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ABSTRACT: *Alzheimer's disease (AD) is the most common cause of dementia in the elderly, and its pathology is characterized by the presence of numerous numbers of senile plaques and neurofibrillary tangles. Several genetic and transgenic studies have indicated that excess amount of β-amyloid protein (Aβ) is produced by mutations of β-amyloid precursor protein and causes learning impairment. Moreover, Aβ has a toxic effect on cultured nerve cells. To prepare AD model animals, we have examined continuous (2 weeks) infusion of Aβ into the cerebral ventricle of rats. Continuous infusion of Aβ induces learning impairment in water maze and passive avoidance tasks, and decreases choline acetyltransferase activity in the frontal cortex and hippocampus. Immunohistochemical analysis revealed diffuse depositions of Aβ in the cerebral cortex and hippocampus around the ventricle. Furthermore, the nicotine-evoked release of acetylcholine and dopamine in the frontal cortex/hippocampus and striatum, respectively, is decreased in the Aβ-infused group. Perfusion of nicotine (50 μM) reduced the amplitude of electrically evoked population spikes in the CA1 pyramidal cells of the control group, but not in those of the Aβ-infused group, suggesting the impairment of nicotinic signaling in the Aβ-infused group. In fact, Kd, but not Bmax, values for [^{3}H] cytisine binding in the hippocampus significantly increased in the Aβ-infused rats, suggesting the decrease in affinity of nicotinic acetylcholine receptors. Long-term potentiation (LTP) induced by tetanic stimulations in CA1 pyramidal cells, which is thought to be an essential mechanism underlying learning and memory, was readily observed in the control group, whereas it was impaired in the Aβ-infused group. Taken together, these results suggest that Aβ infusion impairs the signal transduction mechanisms via nicotinic acetylcholine receptors. This dysfunction may be responsible, at least in part, for the impairment of LTP induction and may lead to learning and memory impairment. We also found the reduction of glutathione- and Mn-superoxide dismutase-like immunoreactivity in the brains of Aβ-infused rats. Administration of antioxidants or nootropics alleviated learning and memory impairment induced by Aβ infusion. We believe that investigation of currently available transgenic and non-transgenic animal models for AD will help to clarify the pathogenic mechanisms and allow assessment of new therapeutic strategies.*

I. INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, and its pathology is characterized by the presence of numerous numbers of senile plaques (SP) and neurofibrillary tangles (Hardy and Allsop, 1991; Kosik, 1991). In AD patients, severe cognitive dysfunction is observed concomitant with neuronal degeneration, particularly in cholinergic neuronal systems (Davies and Maloney, 1976; Whitehouse *et al.*, 1982; Wilcock *et al.*, 1982; Coyle *et al.*, 1983). Since central cholinergic neuronal system plays an important role in learning and memory process (Nabeshima, 1993), it has been thought that cholinergic degeneration is responsible for the impairment of learning and memory in AD (Sims *et al.*, 1983; Bierer *et al.*, 1995).

Although the pathophysiology of AD has been investigated by the numerous numbers of researches of all over the world, several issues remain to be elucidated. One of these unconfirmed things is the mechanisms of neurodegeneration-how and why does neurodegeneration occur? The SP, one of the most characteristic features of AD, mainly consists of β-amyloid protein (Aβ) (Hardy and Allsop, 1991), and several genetic and transgenic studies have indicated that an

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excess amount of Aβ is produced by mutations of β-amyloid precursor protein (APP) (Chartier-Harlin *et al.*, 1991; Goate *et al.*, 1991; Citron *et al.*, 1992; Cai *et al.*, 1993) and causes learning impairment (Murrell *et al.*, 1991; Hsiao *et al.*, 1996). Moreover, Aβ has a toxic effect on cultured nerve cells (Yankner *et al.*, 1989). Therefore, one hypothesis-accumulation of Aβ is responsible for neurodegeneration in AD-has been proposed. The possible mechanisms of neurodegeneration induced by Aβ are

1) disturbance of intracellular ionic balance (Mattson *et al.*, 1993; Etcheberrigaray *et al.*, 1993, 1994).

2) generation or formation of free-radical species (Behl *et al.*, 1994).

3) induction of inflammatory response due to overproduction of cytokines (Meda *et al.*, 1995, 1996), etc.

However, the details of mechanisms underlying neurodegeneration are still unknown.

So far, only a few cholinesterase inhibitors (ChEI) such as tacrine, rivastigmine and donepezil have been developed as effective therapeutic drugs for AD. This is due to the lack of suitable animal models of AD. For instance, animals with electrical or chemical lesions of cholinergic neurons in the basal forebrain show AD-like cognitive dysfunction, but they are not suitable for investigating causes and development of AD. Moreover, they did not show any pathological changes observed in AD patients. It is necessary to develop an adequate animal model of AD. Although APP transgenic mice are useful models of AD to elucidate the processes of Aβ deposition and neuronal degeneration, it takes about a year to develop the learning and memory deficits in these mutant mice (Hsiao *et al.*, 1996; Nalbantoglu *et al.*, 1997).

Therefore, we have tried to develop non-transgenic AD model animals by directly infusing Aβ into the cerebral ventricles of rats. The toxicity of Aβ infusion was evaluated by behavioral, neurochemical and electrophysiological methods. Further, we evaluated some chemicals including antioxidant and cognitive enhancers as possible therapeutics for AD by using this model. In this article, we summarize our results.

II. PREPARATION OF AD ANIMAL MODEL BY INFUSING Aβ

Male Wistar rats (280~320 g) were infused synthetic human Aβ (1-40 or 1-42) dissolved in 35% acetonitrile/0.1% trifluoroacetic acid into the cerebral ventricle. Continuous infusion of Aβ was maintained by a modified osmotic minipump (Alzet 2002; Alza, CA) (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994). As shown in Fig. 1, we used the needle (30G, I.D. 0.30 mm) for insertion into the brain, which was cut to about 2 cm long. The polyethylene tube (inner tube: I.D. 0.28 mm, O.D. 0.61 mm) was cut to 4~5 cm (this length was changed according to the animal size), and one end was connected to the needle and the other end to the ALZET Pump Flow Moderator. Further, the needle-tube-moderator was packed by other tube (outer tube: I.D. 0.76 mm, O.D. 1.22 mm) to avoid the damage and disconnection, because the inner tube is very fine. The rat anesthetized with sodium pentobarbital was fixed in the stereotaxic apparatus. The head skin was cut and skull was exposed and a small hole is drilled. The needle was inserted through this small hole and fixed with dental cement. The pump was implanted under back skin near the blade born. Control group was infused with vehicle (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997; Itoh *et al.*, 1996, 1999; Tanaka *et al.*, 1998; Yamada *et al.*, 1998) or $A\beta_{40\text{-}1}$ (Yamada *et al.*, 1999b, c, d) instead of $A\beta_{1\text{-}40}$ or $A\beta_{1\text{-}42}$.

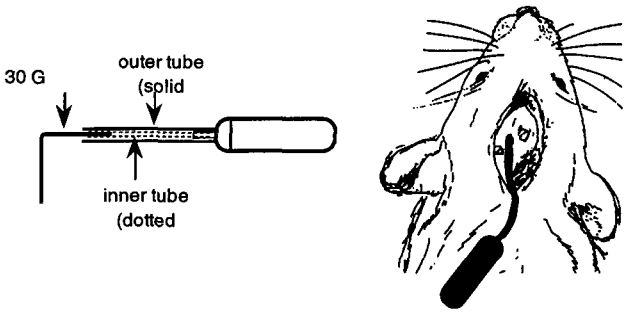

Fig. 1. Schematic drawings of modified osmotic minipump to investigate chronic toxicity of Aβ.

III. EVALUATION OF IMPAIRMENT OF MEMORY IN Aβ-INFUSED RAT

1. Morris's Water Maze Task

First, we investigated several doses of Aβ (3, 30 and 300 pmol/day) to find the optimal dose(s). Between 9 and 13 days after start of infusion, learning ability of

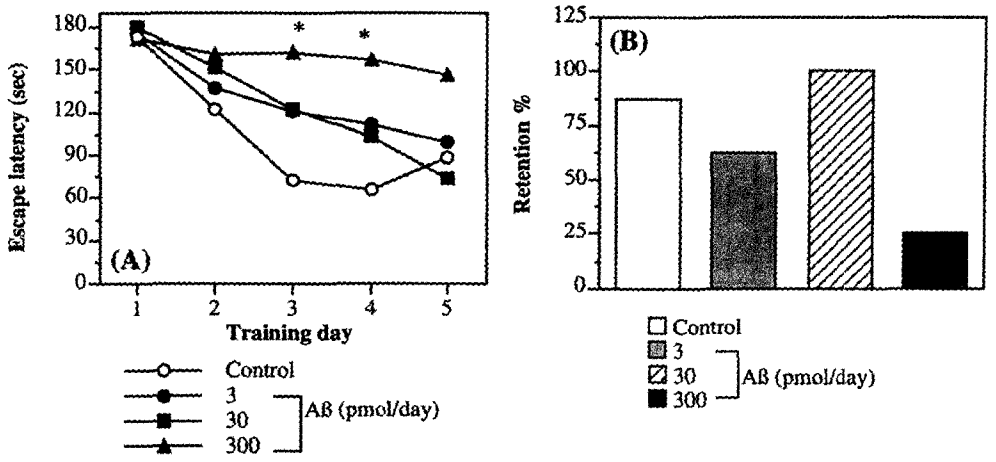

Fig. 2. Effects of continuous Aβ infusion on the performance in Morris's water maze (A) and passive avoidance (B) tasks. Water maze and passive avoidance tasks were carried out 9-13 and 14-15 days after the start of Aβ infusion, respectively. (A) Each value represents the mean of escape latency. *$p<0.05$ vs. control (Tukey's test). (B) Retention % is the percentage of animals per group that showed a step-through latency of 300 sec or more. $\chi^2 = 11.551$, $\alpha = 0.0091$.

rats was examined on Morris's water maze test (Morris, 1984). When the rat failed to find the hidden platform in a 90 sec observation period, the training was terminated and a maximum score of 90 sec was assigned. Training was carried out twice a day for 5 consecutive days (2 trials x 5 days) (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997).

As shown in Fig. 2A, the escape latencies of the Aβ-infused groups in the first training period were not different from those of the control group. Repeated training gradually shortened the escape latencies in the control group, whereas, Aß-infused (particularly 300 pmol/day) groups needed longer time to find the platform compared to the control (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997). Since we could not find the differences on the swimming speed among the groups, it is unlikely that the differences of the escape latency were due to the motor incoordination. Furthermore, Aβ-infused animals shows impaired performance in the probe trial by which the platform was removed from the pool. Performance in the probe trials is a reliably assesses measure of spatial reference memory (Yamada *et al.*, 1999b, c).

2. Passive Avoidance Task

After water maze experiment (14 and 15 days after start of infusion), passive avoidance task was carried out. We employed step-through type passive avoidance task in this experiment (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997). The test criterion was whether the rat remained in the light compartment for at least 300 sec in the retention test. The results were expressed as percentage of animals per group showed a step-through latency (STL) of 300 sec or more (retention %). No significant difference in STL was observed in the acquisition trial (data not shown). As shown in Fig. 2B, the percentage retention of the Aβ-infused (300 pmol/day) group was smaller than that of the control group (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997).

IV. EVALUATION OF NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL TOXICITY IN Aβ-INFUSED RAT

1. Activity of Choline Acetyltransferase (ChAT) and Cholinesterase (ChE) in the Brain of Aβ-infused Rat

Measurement of ChAT and ChE activity, the details of which were reported previously (Ellman *et al.*, 1961; Kaneda and Nagatsu, 1985), was carried out after the behavioral experiment (16 days after start of infusion) (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997).

ChAT activity in the frontal cortex, parietal cortex, striatum and hippocampus in the control group was 1196.4±22.4, 718.9±45.3, 4041.7±691.6 and 899.7± 56.9 nmol/min/g protein, respectively. In Aβ-infused

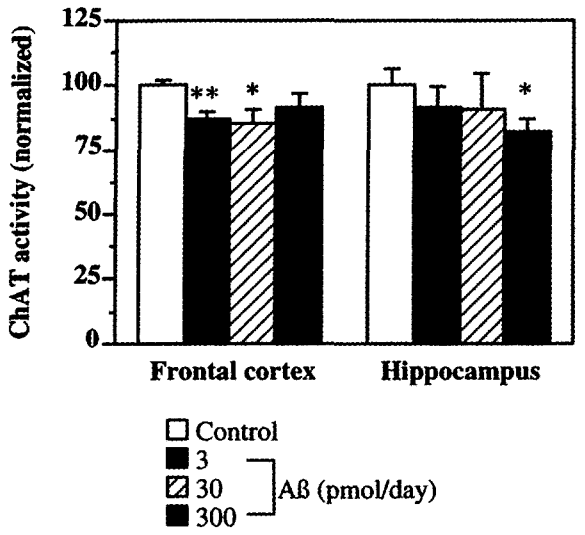

Fig. 3. Effects of continuous Aβ infusion on ChAT activity. Rats were decapitated and the brains were removed for ChAT assay 16 days after the start of Aβ infusion. Each column is expressed as a percentage of the control value and represents the mean±SEM. *p<0.05, **p<0.01 vs. control (Tukey's test).

group, ChAT activities in the frontal cortex (3 and 30 pmol/day) and hippocampus (300 pmol/day) were significantly decreased (Fig. 3), while the activity in the parietal cortex and striatum did not change (data not shown). In the area where decrease of ChAT activity was observed, we also found the diffuse deposition of Aβ confirmed by immunohistochemical staining (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994). ChE activities in the frontal cortex, parietal cortex, striatum and hippocampus in the control group were 1018.9± 35.9, 943.4±52.6, 9353.9±1199.9 and 1350.0±64.0 μmol/hr/mg protein, respectively. There were no significant differences in ChE activities between the control and Aβ-infused groups in any brain regions examined (Nitta *et al.*, 1994, 1997).

Since the most effective dose of Aβ was 300 pmol/day, therefore, in the following experiments the dose of Aβ was fixed at 300 pmol/day to investigate Aβ toxicity.

2. [^{3}H] Cytisine Binding in the Brain of Aβ-infused Rats

Receptor binding assay was performed as described by Rowell and Li (1997). On the 19th days, after the start of the β-amyloid infusion, the rats were killed by decapitation. The brains were quickly removed from the skull and the cerebral cortex, hippocampus and striatum were immediately dissected out.

Bmax values for [^{3}H] cytisine binding in the cerebral cortex, hippocampus and striatum of the Aβ-infused rats did not differ from those of the control. No change was also observed on Kd values in the striatum, however, Kd value in the hippocampus significantly increased in the Aβ-infused rats and that in the cerebral cortex tended to increase. These results suggest that the brain dysfunction induced by continuous infusion of Aβ may be due to the decrease of affinity of nAChR (Olariu *et al.*, 1999).

3. Changes of Oxidative Stress-related Enzyme Activity

Based on the *in vitro* findings that free radical and oxidative stress play an important role in Aß-induced neurodegeneration (Behl *et al.*, 1994), we examined the changes in expression of oxidative stress-related enzyme (Mn-superoxide dismutase (SOD-2), glutathion (GSH), GSH-S-transferase-P (GSTP) and GSH-peroxidase (GPX)) in Aβ-infused rats. SOD-2-like immunoreactivity (SOD-2-IR) in the parietal cortex was reduced in Aβ-infused group, but not in control group (Im *et al.*, 1999). Moreover, SOD-2-IR in the substantia nigra was disappeared without any neuronal loss (as evaluated by tyrosine hydroxylase immunoreactivity and cell body staining) in Aß-infused group. The reduction of GSH-IR was found in Ammons horn of the hippocampus and the thalamic area in Aβ-infused group (Jhoo *et al.*, 1999). Similar to GSH-IR, GSTP-IR significantly decreased in the thalamic and cortical area and the reduction of GPX-IR was mainly observed in the cortical neurons (Jhoo *et al.*, 1999). These results suggest that reduction of enzyme expression that protect from oxidative stress is involved in Aβ-induced neurodegeneration.

4. *In vivo* Brain Microdialysis

To evaluate the toxicity of Aβ on neurotransmitter release, in vivo brain microdialysis was also performed for acetylcholine (ACh) and dopamine (DA) release in the frontal cortex/hippocampus and striatum, respectively (Itoh *et al.*, 1996).

Ten to 12 days after the start of Aβ infusion, the

cannula delivering Aβ was removed and a dialysis probe was implanted. Briefly, a dialysis probe was implanted into a region extending from the frontal cortex to hippocampus (A -3.5; L 2.0; V 1.0~4.0 mm) for ACh and the striatum (A -0.5; L 3.0; V 4.0~7.0 mm) for DA (Paxinos and Watson, 1986). About 20 h after implantation of the dialysis probe, Ringer's solution was perfused. The amount of ACh and DA in collected dialysate was detected by HPLC system with electrochemical detection.

First, we compared the nicotine-evoked ACh and DA release between the control and Aß-infused group because it has been demonstrated that nicotine enhances several neurotransmitters release *via* presynaptic nicotinic ACh receptors (nAChR) (Brazell *et al.*, 1990; Nordberg *et al.*, 1989; Wonnacott, 1990). After ACh and DA levels in dialysate became stable (we could not find significant differences on basal ACh and DA release between the control and Aβ-infused group), Ringers solution containing 3 mM nicotine (NIC-Ringer) in place of normal Ringer was perfused. NIC-Ringer was perfused for 30 min and 1 h for ACh and DA, respectively, and then replaced with normal Ringer.

As shown in 4A, the extracellular ACh level in the frontal cortex/hippocampus was elevated to about 2-fold by perfusion of NIC-Ringer and returned to the basal level 60 min after the cessation of the perfusion in the control group. In the Aβ-infused group, however, the nicotine-evoked release of ACh was significantly lower than that in the control group. Perfusion of NIC-Ringer also increased the extracellular DA level by 1.5-fold in the striatum of the control group, and the effect disappeared by 30 min after the cessation of the perfusion (Fig. 4B). In contrast, nicotine failed to increase the extracellular level of DA in the Aß-infused group (Itoh *et al.*, 1996)

About 3 h after NIC treatment, the levels of both transmitters returned to basal level, and then high K Ringer's solution (51 mM NaCl, 100 mM KCl, 1.25 mM $CaCl_2$; high K-Ringer) was perfused for 15 min and 30 min for ACh and DA, respectively. The perfusion of high K-Ringer increased the ACh level to approximately 4-fold in the frontal cortex/hippocam-

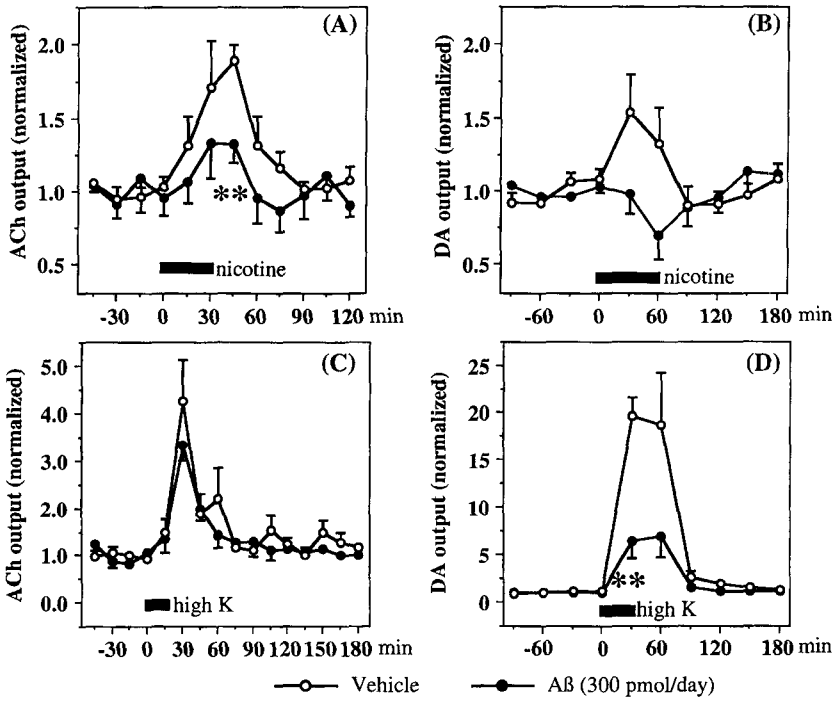

Fig. 4. Effect of nicotine and high K on extracellular ACh level in the frontal cortex/hippocampus (A: nicotine, C: high K) and DA level in the striatum (B: nicotine, D: high K) of control and Aβ-infused (300 pmol/day) rats. Rats were implanted with a microdialysis probe 10-12 days after the start of infusion, and the next day, dialysis was performed. Nicotine-Ringer was perfused for 30 min (A) and 1 hr (B) and high K-Ringer was perfused for 15 min (C) and 30 min (D), after which normal Ringer's solution was perfused. Each value represents the mean±SEM. (A) [$F_{(1,92)}$ = 6.866, $p<0.05$ (2-way ANOVA); **$p<0.01$ vs. control (Scheffe's test), (B) [$F_{(9,64)}$ = 2.267, $p<0.05$ (2-way ANOVA)], (D) [$F_{(1,79)}$ = 16.43, $p<0.01$, **$p<0.01$ (2-way ANOVA)].

pus of the control group. Although the magnitude of the increase in the Aβ-infused group was less than that in the control group, there was no statistical difference between the control and the Aβ-infused groups (Fig. 4C). A dramatic increase (more than 15-fold) in the extracellular DA level was induced in the control group by the perfusion of high K-Ringer in the striatum. The high K-evoked DA release in the Aβ-infused group was significantly lower than in the control group (Fig. 4D) (Itoh *et al.*, 1996).

These results suggest that decrease of neurotransmitter release may be responsible, at least in part, for learning and memory impairment induced by Aβ infusion.

5. Electrophysiological Analysis

Ten or 11 days after the start of Aβ infusion, 400 μm-thick brain slices of the hippocampus were prepared for extracellular recordings. Electrical stimulation was applied in the radiatum-lacunosum layers to stimulate Shaffer collaterals and/or commissural fibers in the CA1 region. A recording glass pipette was placed in the pyramidal cell layer near the stimulating electrode. The intensity of the test stimuli was adjusted to evoke about 50% of the maximum response. Stimuli were given every 30 sec. To examine the effects of nicotine, nicotine was added into perfusing solution (50 μM) for 10 min followed by normal perfusing solution (Itoh *et al.*, 1999).

Application of nicotine into the perfusing solution decreased the population spike (PS) amplitude in hip-pocampal CA1 pyramidal cells of the control group (Fig. 5A). Five min after the application of nicotine, the PS amplitude decreased to approximately half the basal level. The PS amplitude returned to the control level 5 min after perfusion without nicotine. The nicotine-induced reduction of PS amplitude was attenuated by co-application of the nicotinic antagonist, mecamylamine (10 μM, data not shown), indicating that the reduction was mediated *via* nAChR. In the Aβ-infused group, the degree of nicotine-induced reduction of PS amplitude was significantly less than that in the control group. This result is consistent with the one obtained in the microdialysis experiments that showed a decrease of nicotine-evoked release of ACh and DA (Itoh *et al.*, 1996). Therefore, it is conceivable that Aβ infusion impairs the function of nAChR, and/or the process of nicotinic signal transduction.

Next, we investigated whether the ability of long-term potentiation (LTP) induction in the Aß-infused group was impaired, since LTP is thought to be an essential mechanism underlying learning and memory (Bliss and Collingridge, 1993).

To induce LTP, a tetanic stimulation (100 Hz for 1 sec, the same intensity during the basal stimulation) was applied (Itoh *et al.*, 1999).

Immediately after the tetanic stimulation, the PS amplitude was greatly enhanced in the control group to about 4-5-fold of the control level (Fig. 5B). The PS amplitude gradually decreased to a level about 2-fold of the control level, which persisted for more than 45 min in the control group (Fig. 5B). In contrast, although a similar degree of enhancement of PS was observed immediately after tetanus in the Aß-infused

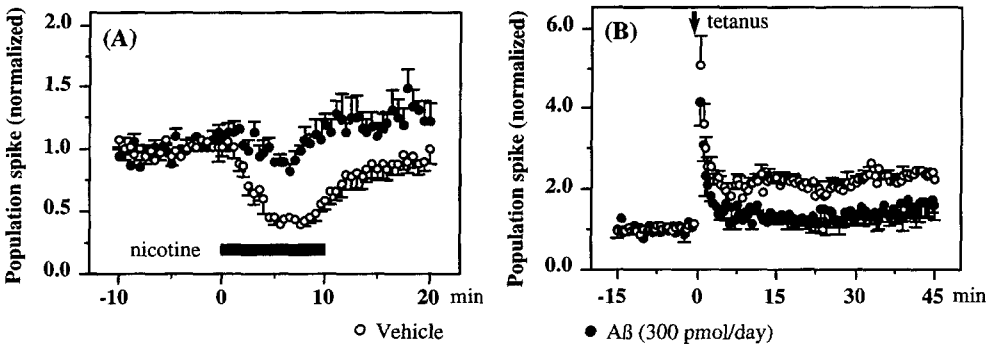

Fig. 5. Effects of continuous infusion of Aβ on the response to nicotine (A) and on the LTP induction (B) in the hippocampal CA1 pyramidal cells. The bar in (A) and the arrow in (B) indicate the time when nicotine (50 μM) and tetanic stimulation (100 Hz, 1 sec), respectively, was applied. (A) [$F_{(1,483)} = 282.422$, $p<0.01$ (2-way ANOVA)], (B) [$F_{(1,1417)} = 833.538$, $p<0.01$ (2-way ANOVA)].

Table 1. Summary for the effects of NC-1900 (an arginine-vasopressin analog), propentofylline, idebenone, α-tocopherol and nefiracetam on β-amyloid-induced learning and memory deficits in rats

Test	Treatment	Aβ (1-40) or (1-42) alone	Aβ (1-40) or (1-42) with									
			NC-1900 (ng/kg, s.c.)		propentofylline (mg/kg, p.o.)		idebenone (mg/kg, p.o.)		a-tocopherol (mg/kg, p.o.)	nefiracetam (mg/kg, p.o.)		
			0.1	1	10	25	10	20	150	1	3	10
Water maze												
	Reference memory	↓↓	±	↑↑	±	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑
	Working memory	↓↓	±	±	n.d.	n.d.	±	↑↑	↑↑	↑↑	↑↑	↑↑
Passive avoidance												
	Acquisition	±	±	±	±	±	±	±	±	±	±	±
	Retention	↓↓	±	↑↑	↑↑	±	±	±	±	↑↑	↑↑	↑↑

↓↓ : Impairment.↑↑ : Improvement. ± : no change. n.d. : not determined.

group, the PS amplitude soon returned to the control level. The PS amplitude of Aβ-infused group after tetanic stimulation was slightly higher than that of their basal level, but it was significantly lower than that of the control group. The PS amplitude at the end of observation period (45 min) of control group was significantly higher than that of Aβ-infused group (Itoh *et al.*, 1999). This result indicates the impairment of LTP induction in the CA1 subfield of the hippocampus in the Aß-infused group.

Since there is general agreement that hippocampus plays an important role in memory processes, this deficiency may be responsible, in part, for learning and memory impairment in the Aß-infused group.

V. EFFECTS OF ANTIOXIDANTS/COGNITIVE ENHANCERS ON Aβ-INDUCED LEARNING IMPAIRMENT

We have also evaluated the effects of several chemicals including antioxidants and cognitive enhancers on Aβ-toxicity.

1. Antioxidants

As mentioned in "Introduction" and above, oxidative stress may be responsible for Aβ-induced neurodegeneration (Behl *et al.*, 1994, Im *et al.*, 1999, Jhoo *et al.*, 1999). To test this ideas, we investigated whether antioxidants such as idebenone (Yamada *et al.*, 1999b) and α-tocopherol (Yamada *et al.*, 1999b) attenuates Aβ-induced learning impairment. These chemicals were administered once a day by orally from 3 days before the start of Aβ infusion until the end of behavioral experiments. The results were summarized in Table 1. These chemicals attenuated Aβ-induced learning impairments especially in the spatial learning of the water maze. These results provide an *in vivo* evidence that oxidative stress is involved in Aβ-induced learning impairments (Yamada *et al.*, 1999b). These antioxidants may prevent learning impairment in the Aβ-infused animal by protecting neurons from free radical-mediated Aβ toxicity (Yamada *et al.*, 1999b).

2. Cognitive Enhancers

Nefiracetam is under development in Japan as a nootropic for the treatment of cerebral vascular dementia and AD (Nabeshima, 1994). It significantly improved the impairment of memory in patients with cerebral vascular dementia in a phase III clinical study and the impairment of performance in animals in various experimental memory tests (Nabeshima, 1994). We evaluated the effect of nefiracetam on the impairment of performance of Aβ-infused animals (Yamada *et al.*, 1999c). We also examined the effects of propentophylline (Yamada *et al.*, 1998) and NC-1900, an arginine-vasopressin derivative (Tanaka *et al.*, 1998), on Aß-induced memory impairment.

As summarized in Table 1, nefiracetam (1~10 mg/kg/day, p.o.) administered 1 h before the behavioral experiments significantly ameliorates learning impairment in Aβ-infused animals. It is noteworthy that repeated oral administration of nefiracetam was commenced 7 days after the start of Aβ infusion. Although its mechanism of action is not completely understood, we speculate that nefiracetam ameliorates the learning impairments induced by Aβ infusion at least in part by activating voltage-sensitive Ca^{2+} channels,

and thereby improving dysfunction of cholinergic and dopaminergic neuronal systems in Aβ-infused animals (Yamada *et al.*, 1999c).

Propentophylline (10 and 25 mg/kg) and NC-1900 (1 ng/kg) administered once a day by orally and subcutaneously, respectively, from 3 days before the start of Aβ infusion also attenuates Aβ-induced learning impairments (Yamada *et al.*, 1998; Tanaka *et al.*, 1998).

VI. CONCLUSION

It has been reported that Aβ toxicity is potentiated by self-aggregation of Aß and that this aggregation is accelerated under physiological conditions (Pike *et al.*, 1991). Under our experimental conditions, it is unlikely that Aβ aggregated in the pump, as we selected a solvent (35% acetonitrile/0.1% trifluoroacetic acid) that would avoid such aggregation. Although acetonitrile toxicity to neuronal cells has been suggested (Waite *et al.*, 1992), our preliminary experiments showed no deteriorating effect on learning ability and ChAT activity. This may be due to the slow infusion rate (0.5 μl/h) or to the infusion site (into the ventricle, not parenchyma). In our experiments, we could not find significant differences in learning ability, neurochemical and electrophysiological measures between the intact and control groups. Based upon these observations, it is likely that the neuronal dysfunctions found in the Aβ-infused rat are induced by Aβ itself but not by the solvent used.

Taken together, we propose the possible mechanisms of neuronal dysfunction induced by the continuous infusion of Aβ into the cerebral ventricle as follows:

Continuous Aβ infusion

1. accumulates Aβ (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997; Yamada *et al.*, 1999a).

2. fails maintenance of ionic balance (Mattson *et al.*, 1993; Etcheberrigaray *et al.*, 1993, 1994) or overproduces free-radicals species (Behl *et al.*, 1994; Im *et al.*, 1999, Jhoo *et al.*, 1999; Yamada *et al.*, 1999b) which induce inflammatory response and damage the neurons (Nitta *et al.*, 1997).

3. impairs signal transduction *via* nAChR (Itoh *et al.*, 1996, 1999, Olariu *et al.*, 1999).

4. decreases neurotransmitter release (Itoh *et al.*, 1996).

5. impairs LTP induction (Itoh *et al.*, 1999) and learning and memory (Nabeshima and Nitta, 1994; Nitta *et al.*, 1994, 1997; Tanaka *et al.*, 1998; Yamada *et al.*, 1998, 1999b, c, d).

These behavioral, neurochemical and physiological changes observed in our model animal were not observed in previous animal model of AD that have been used to examine the efficacy of the candidates of cognitive enhancers or nootropics for AD. The animal models that precisely exhibit the pathophysiology are very important when estimate the drugs whether they were actually effective or not. This gap in pathophysiology between animal models and AD patients retard the development of actually useful therapeutics. Recent remarkable progression of biotechnology provided animal models artificially modified the disease-related gene, and they show similar pathophysiology observed in the patients. With regard to AD, transgenic (APP, preseniline 1, 2) models have been reported (Citron *et al.*, 1998; Duff *et al.*, 1996; Hsiao *et al.*, 1996; Nalbantoglu *et al.*, 1997). However, these models might not be freely available in every laboratory and time consuming to get aging animals.

The method of preparation of AD model animals by continuous Aß infusion is convenient and is useful for screening the chemicals, which may have potentials as AD therapeutic drugs. At present, activation or enhancement of survived neurons is main current in the development of AD therapeutic drugs. Actually, only one ChE inhibitor is available for treatment of AD in Japanese clinic. Therefore, we focused on chemicals such as propentofylline and idebenone, since they have neuroprotective property, have been focused. It has been reported that propentofylline and idebenone enhances nerve growth factor (NGF) secretion (Nabeshima *et al.*, 1993; Nitta *et al.*, 1993), which is essential for cholinergic neuron in basal forebrain to survive (Yu *et al.*, 1978). It may be possible to enhance the survival of cholinergic neurons by NGF. The effectiveness of NGF in AD patients has been reported by Eriksdotter-Jonhagen *et al.* (1998). In this case, however, NGF was administered directly into patients brain because NGF can not pass the blood-brain barrier. This method is not recommended from a point of view of "quality of life". So, the developments of chemicals such as idebenone,

which can be administered peripherally, are expected as AD therapeutic drugs. We have noted that there are cases in which our method is not suited, because accumulation or deposition of Aβ in this model is not physiologically. For researching the process of Aβ deposition and toxicity and assessing the effects of drugs that inhibit Aβ synthesis, fibril formation and deposition in the brain, transgenic models give full play to their ability.

We believe that investigation of currently available transgenic and non-transgenic animal models for AD will help to clarify the pathogenic mechanisms and allow assessment of the effects of new therapeutic strategies.

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