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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

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Summary. In the present study we found that chronic infusion of β-amyloid fragment (25–35) at nanomolar concentration into rat cerebral ventricle impairs learning and memory. At a concentration of 3 nmol/day but not 0.3 nmol/day, β-amyloid significantly reduced the spontaneous alternation behavior and the memory performance in the water maze and multiple passive avoidance tests. A significant increase in anxiety was also found in the animals infused with 3 nmol/day β-amyloid fragment. Memory deficits and the increased emotionality were correlated with a decreased nicotine-evoked acetylcholine release from the frontal cortex/hippocampus, as assessed by microdialysis, in freely moving rats. The amyloid fragment infused either at pico- or nanomolar concentrations reduced the affinity of [^{3}H] phorbol dibutyrate binding, an index of activated protein kinase C (PKC), and increased the total number of binding sites in the hippocampal particulate fraction. Our results suggest that the amnesic and anxiogenic effects of chronic infusion of β-amyloid (25–35) are related to the decreased acetylcholine release and reduced PKC activation.

Keywords: Memory impairment, emotional behavior, β-amyloid, cholinergic deficit, protein kinase C (PKC).

Introduction

β-Amyloidogenesis is thought to be critical in the pathogenesis of Alzheimer's disease (AD) (Selkoe, 1994, 1996). The two major forms of the aggregated β-amyloid peptide comprising 40 or 42 amino acids have been well described as the prominent components of the senile plaques in AD. Accumulation of the peptides is associated with the progressive neuronal death, cognitive deficits

and neuropsychiatric disorders such as agitation, apathy and an increased anxiety (Hardy et al., 1992; Selkoe, 1996; Scheuner et al., 1996; Weiner et al., 1997). The neurotoxic properties of the β-amyloid peptides (1–40) or (1–42) were mimicked in vitro by the core amino acid sequence (25–35) of the amyloid peptide (Yankner et al., 1990). Since then, the short fragment of β-amyloid has been of a special interest and a variety of in vitro studies which attempting to model the AD pathology used the peptide to examine the neurotoxic properties of the β-amyloid proteins. Although it is not clear whether or not the amyloid fragment (25–35) occurs in the brain of AD individuals, it has been described that the acute injection of the peptide into rat cerebral ventricle exerts neurotoxic effects similar with those produced by the β-amyloid (1–40) (Kowall et al., 1991; Yamada and Nabeshima, 2000). Limited reports indicated also memory deficits induced by the amyloid fragment when it was injected into the cerebral ventricle or locally into the hippocampus of rodents (Dornan et al., 1993; Maurice et al., 1996). So far there are no available data related to the effect of the amyloid fragment (25–35) on the emotional behavior. It is also questionable whether or not the concentration of β-amyloid which demonstrates the amnesic effects in rodents is prone to induce also an anxiogenic symptom.

Therefore, in the present study we consider to analyze the impact of chronic infusion of β-amyloid (25–35) into the rat cerebral ventricle, on the performance in a variety of learning tasks as an index of the amnesic potency of the peptide and on the elevated plus maze task as a measure of the anxiety status in animals. In our attempt to find the neurochemical substrate of the behavioral changes induced by the amyloid treatment we directly measured the extracellular acetylcholine (ACh) release in the frontal cortex/hippocampus of animals treated with the amyloid peptide and detected also the level of activated protein kinase C (PKC) in the hippocampal membrane fractions.

Materials and methods

Animals and treatment

Male Wistar rats (Charles River Japan, Yokohama, Japan) weighing 200–225 g at the beginning of the experiments were used. They were housed two or three per cage under standard light-dark conditions (12 h light cycle starting at 9:00 am) at a constant temperature of 23°C. The animals had free access to food and water and they have been handled in accordance with the guidelines established by the Institute for Laboratory Animal Research of Nagoya University.

On the day of surgery, a cannula attached to a mini-osmotic pump was implanted in the rat right cerebral ventricle (A – 0.3 mm, L 1.2 mm, V 4.5 mm) as previously described (Nitta et al., 1994) and β-amyloid (25–35) (Feinchemikalien AG, Switzerland) was continuously infused at doses of 0.3 or 3 nmol/12 μl/day for at least 14 days. Control animals received only vehicle (35% acetonitrile/0.1% trifluoroacetic acid). We have previously confirmed that either β-amyloid (40–1) or vehicle itself has no effect on learning behavior at this flow rate (Nitta et al., 1994; Yamada et al., 1998, 1999a,b). On the 18th day after the start of 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, kept on dry ice and subsequently stored at −80°C until assayed.

Locomotor activity

The measurement of the locomotor activity was carried out on day 7 after the start of β-amyloid infusion in a session of 10 min. The apparatus consisted of a locomotor cage (25 × 42 × 20 cm), with photobeams placed 2 cm above the floor at 1-in. intervals along two sides of the cage (Colombus Instruments, USA).

Y-maze task

The performance in the Y-maze test, a measure of the spatial and short-term memory was carried out on day 8 after the start of β-amyloid infusion (Yamada et al., 1996; Hsiao et al., 1996). The apparatus was made of gray-painted plywood. Each arm was 35 cm long, 25 cm high and 10 cm wide and positioned at equal angle. The rat was placed at the end of one arm and allowed to move freely through the maze for a 8 min session. Spontaneous alternation behavior was defined as the entry into all three arms on consecutive three choices in overlapping triplet sets. The percent spontaneous alternation behavior was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries – 2) ×100.

Elevated-plus-maze

A wooden plus maze consisting of opposite pairs of open and enclosed arms of equal length and width (50 × 10 cm) was used (Miyagawa et al., 1998). The enclosed arms had wooden walls of 40 cm high. The maze was set up 50 cm above the ground. Experiments were carried out on day 9 after the start of the infusion in a sound attenuated room with low indirect illumination (44 lux). Plus maze performance was assessed by the percentage of open arm entries (open arm entries = number of open arm entries/total number of arm entries × 100) and open arm time (open arm time = time spent in open arm/total time × 100). An arm entry was defined as the invasion of the arm with the four paws.

Water maze test

The water maze task was carried out on day 10 to 15 after the start of β-amyloid (25–35) infusion (Morris, 1984; Nitta et al., 1994). The swimming pool consisted of a circular water tank (140 cm in diameter and 45 cm high). A transparent platform (12 cm diameter and 25 cm high) was placed 2 cm bellow the surface of water. The water temperature was kept approximately at 23°C. The pool was located in a room with external cues such as pictures and lamps. Spatial learning and memory was assessed by subjecting the animals to two trials per day for five consecutive days (total ten trials). For each training trial, the rat was placed in the water faced to the pool wall at one of five starting positions that were chosen randomly. The position of the platform was unchanged during the reference memory task. The latency to escape onto the hidden platform was recorded for 90 sec. If the rat found the platform within 90 sec, another 15 sec allowed him to remain on the platform and then he has been returned to the home cage. In the case that the rat failed to find the platform, the training session was terminated. A score of 90 sec was assigned and the animal was guided by the experimenter to find the platform and allowed to remain on it for 15 sec.

Immediately after the 10th trial, the platform was removed from the pool and the animals were tested on a spatial probe trial. The percentage of time spent in the platform quadrant was recorded for 90 sec.

Twenty-four hours after the probe trial, the rats were subjected to a reversal learning test consisting of two consecutive trials. The platform was placed in the opposite quadrant as compared with the initial learning task. The escape latency to find the platform was recorded as described above.

Multiple-trial passive avoidance test

Multiple-trial passive avoidance test was carried out on days 16–17 after the start of β-amyloid infusion (Yamada et al., 1998). The apparatus consisted of two compartments (25 × 15 × 15 cm high), one illuminated and one dark, both equipped with a grid floor. The two compartments were separated by a guillotine door. In the acquisition trial, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable footshock (0.3 mA, 5 sec) was delivered through the grid floor. The rat was removed after receiving the footshock and was placed back into the light compartment by the experimenter. The door was again opened 30 sec later to start the next trial. Training continued in this manner until the rat stayed in the light compartment for 120 s on a single trial. In the retention trial, given 24 hours after the acquisition test, the rat was placed in the illuminated compartment and the time until it entered the dark compartment was measured as a step-through latency. When the rat did not enter for at least 300 sec, a score of 300 sec was assigned. Nociceptive property of footshock was equally perceived by the β-amyloid- and vehicle-treated animals. Vocalizing, jumping and running were similar in all groups.

[^{3}H]PDBu binding

The phorbol dibutyrate (PDBu) binding was performed as described previously (Gleiter et al., 1988). Frozen brain tissue of cerebral cortex, hippocampus and striatum was homogenized in ice-cold 50 mM Tris-HCl (pH = 7.4) by using a Polytron for 30 sec and centrifuged 27,000 × g for 20 min at 4°C. The pellet was resuspended in the same buffer at a concentration of 15 mg tissue/ml and kept at −80°C until use. The assay was performed in polypropylene tubes (duplicate) in a total mixture volume of 250 µl. Tissue homogenate (7.5 mg tissue/ml) was incubated with four different concentration of [^{3}H]PDBu (ranging from 3.75 to 30 nM, specific activity 740 GBq/mmol, NEN, Boston, MA) for 30 min at 25°C. [^{3}H]PDBu was prepared in buffer containing 0.1% BSA in order to stabilize free phorbol ester concentrations during incubation and dilution (Dunphy et al., 1980). Non-specific binding was determined in the presence of 10 µM cold PDBu. The incubation was stopped by addition of 3 ml ice-cold Tris-HCl (50 mM) and rapidly washed three times through GF-B filters (presoaked in 0.3% polyethylenimine) mounted on a Brandel cell harvester. The filters were dried, 5 ml of Scintisol solution were added and the radioactivity on the filters was determined by a scintillation counter. Protein concentration was measured following the method of Lowry (Lowry et al., 1951), using bovine serum albumin as standard.

Determination of ACh release

In a second group of rats, ten days after starting the infusion of β-amyloid (25–35) either at 0.3 or 3 nmol/day, the i.c.v. cannula was removed and a dialysis probe was implanted for measurement of ACh release (Itoh et al., 1996). Briefly, a dialysis probe (A-I-8-03; membrane length 3 mm, EICOM, Kyoto, Japan) was implanted into a region extending from the fronto-parietal cortex to the hippocampus [AP −3.8; L 2.2; V 1.0–4.0 mm; (Paxinos and Watson, 1986)], since deposition of amyloid was observed in the frontal cortex and hippocampus around the ventricle (Nabeshima and Nitta, 1994; Nitta et al., 1994). Twenty-four hr after the implantation of the dialysis probe, Ringer's solution (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) containing 10^{-5} M eserine was perfused at a flow rate of 2.0 µl/min. The dialysate was collected every 15 min and ACh in the dialysate was determined by an HPLC system with electrochemical detection (ECD). Details of measurement of ACh by HPLC-ECD have been described previously (Hasegawa et al., 1993). After the basal release of ACh had been reached, nicotine (free base, 3 mM) was infused for 30 min.

Statistical analysis

The results are expressed as means ± SEM and were subjected to one way analysis of variance (ANOVA). Post-hoc comparisons between groups were made using Bonferroni test. In the water-maze task the data were analyzed by using two-way ANOVA with β-amyloid treatment and trial as factors. In the elevated-plus-maze test the data were subjected to ANOVA followed by Dunnet post-hoc test. The results of acetylcholine release were compared using the Wilcoxon test followed by Bonferroni as a post-hoc test. The values of $p < 0.05$ were considered significant.

Results

Effect of β-amyloid (25–35) on locomotor activity

The chronic infusion of β-amyloid fragment into cerebral ventricle in rat at concentrations of 0.3 or 3 nmol/day has no significant effect on locomotor activity measured during a 10-min session on day 7 after the start of β-amyloid (25–35) infusion ($p = 0.07$). The locomotor activity counts in vehicle, 0.3 and 3 nmol/day β-amyloid were 1,493 ± 125, 2,031 ± 184 and 1,607 ± 172, respectively.

Effect of β-amyloid (25–35) on Y-maze performance

Spontaneous alternation behavior in the Y-maze test, a measure of the short-term memory, was tested on day 8 after the start of β-amyloid infusion. β-Amyloid (25–35) at the concentration of 3 nmol/day significantly reduced the spontaneous alternation behavior (64.8 ± 3.2% vs 77.8 ± 3.3% for vehicle; $p < 0.01$). The total number of arm entries was not significantly different among groups (Fig. 1).

Anxiogenic-like effects of β-amyloid (25–35) on elevated-plus maze task

The emotional behavior of the animals treated with β-amyloid fragment (25–35) was investigated on day 9 after the start of β-amyloid infusion. As shown

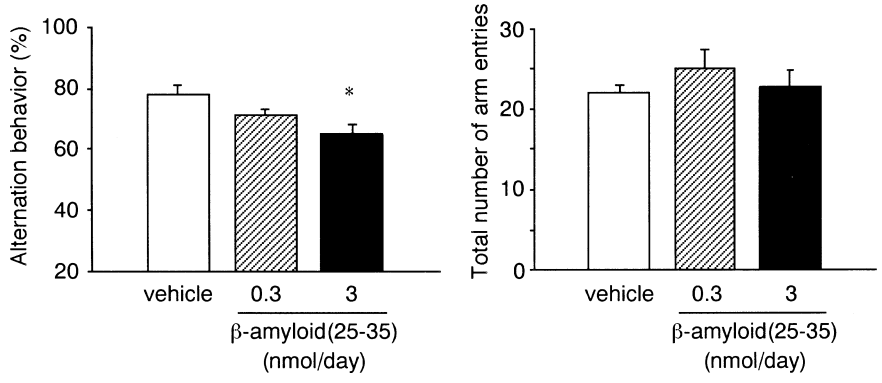

Fig. 1. Effect of β-amyloid (25–35) on the Y-maze performance tested on day 8 after the start of the infusion. The values represent the means ± SEM. The number of the animals in each group were for vehicle = 10, 0.3 nmol/day β-amyloid = 10, and 3 nmol/day β-amyloid = 11. *$p < 0.05$ vs. vehicle

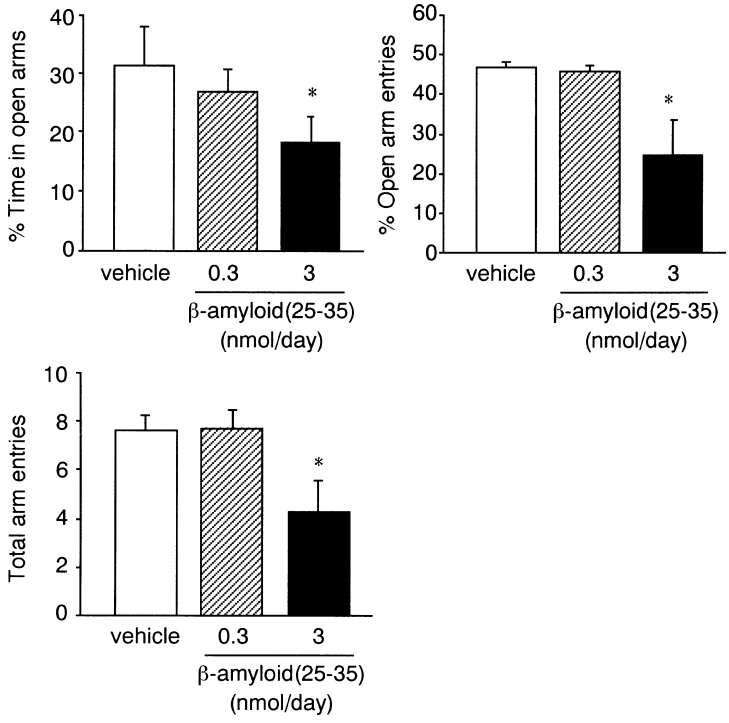

Fig. 2. Effect of β-amyloid (25–35) on the elevated-plus maze on day 9 after the start of the infusion. The values represent the means ± SEM. The number of the animals in each group were for vehicle = 10, 0.3 nmol/day β-amyloid = 10, and 3 nmol/day β-amyloid = 11. *$p < 0.05$ vs. vehicle

in Fig. 2, percentage of the time spent in open arms was significantly decreased when compared with vehicle group (18.2 ± 4.5% vs. 31.2 ± 6.8%, $p < 0.05$). There was also a significant reduction in the percentage number of entries onto the open arms by rats infused with 3 nmol/day β-amyloid (24.9 ± 8.9% vs. 46.8 ± 1.6%, $p = 0.0027$) as well as in the total number of arm entries (4.3 ± 1.3 vs. 7.6 ± 0.6, $p = 0.027$). On the other hand, administration of β-amyloid at the concentration of 0.3 nmol/day did not significantly affect the emotional behavior when compared with the vehicle effect itself.

Effect of β-amyloid (25–35) in the water maze task

Spatial learning and memory in β-amyloid-treated animals was tested in the water maze task on day 10 to 16 after the start of β-amyloid infusion. Comparison of the β-amyloid treated groups and group receiving vehicle revealed that performance in the water-maze task did not significantly altered during the first six training trials (Fig. 3). In the last four trials, the animals infused with 3 nmol/day β-amyloid (25–35), showed a tendency having an increased latency to escape onto the hidden platform. The reference memory impairment was evident in the probe trial, assessed immediately after the last training trial: A significant decrease in the time spent in the platform quadrant was found in the animals infused with β-amyloid peptide at both concentrations

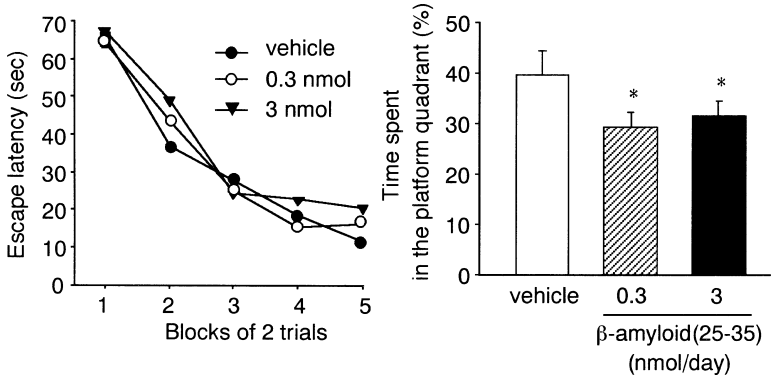

Fig. 3. Reference memory performance in water-maze task carried out on days 10–14 after the i.c.v. infusion of β-amyloid (25–35). The panel inserted shows the performance in the probe trial measured for 30 sec, on day 14 after the infusion. The values represent the means ± SEM. The number of the animals in each group were for vehicle = 10, 0.3 nmol/day β-amyloid = 10, and 3 nmol/day β-amyloid = 11. *$p < 0.05$ vs. vehicle

($p < 0.01$) (Fig. 3). In the reversal learning test measured 24 hours after the probe trial, there was no difference in escape latency in the first trial (Trial-1) among three groups. However, a significant increase in latency to find the platform located at a new position was evident in rats treated with 3 nmol/day β-amyloid (25–35) ($p < 0.01$, Fig. 4).

Effect of β-amyloid (25–35) in the multiple-trial passive avoidance test

Long-term memory was tested in the multiple-trial passive avoidance paradigm on day 17 to 18 after the start of β-amyloid infusion. The step-through latencies in the retention trial carried out 24 hours after the acquisition session was decreased in the rats infused with β-amyloid at the concentration of 3 nmol/day (132.2 ± 34.8 sec), when compared with the vehicle-treated group

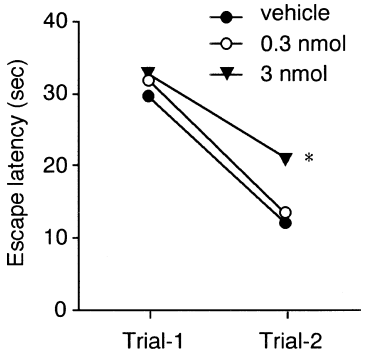

Fig. 4. Reverse learning task carried on day 15 after the i.c.v. infusion of β-amyloid (25–35). The values represent the means ± SEM. The number of the animals in each group were for vehicle = 10, for 0.3 nmol/day β-amyloid = 10, and for 3 nmol/day β-amyloid = 11. *$p < 0.05$ vs. vehicle

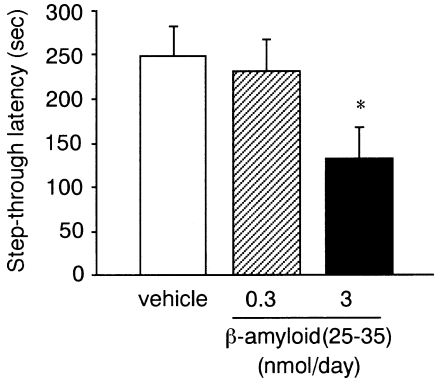

Fig. 5. Reduced step-through latency in the multiple passive avoidance test performed on days 16–17 after the start of β-amyloid (25–35) infusion. The values represent the mean ± SEM. The number of the animals in each group were for vehicle = 10, for 0.3 nmol/day β-amyloid = 10, and for 3 nmol/day β-amyloid = 11. *$p < 0.05$ vs. vehicle

(249.9 ± 33.9 sec) ($p < 0.05$) (Fig. 5). No significant effect was found in the step-through latencies or in the number of trials among groups during the acquisition test (data not shown).

Modulation of [^{3}H]PDBu binding by β-amyloid (25–35)

Chronic infusion of β-amyloid (25–35) at both concentrations (0.3 or 3 nmol/day) into cerebral ventricle significantly increased the dissociation constant (kD) values for [^{3}H]PDBu binding in hippocampal membrane fraction (20.5 ± 0.59 nM for 0.3 nmol/day group, 21.6 ± 1.23 nM for 3 nmol/day group, and 16.86 ± 1.59 nM for the vehicle; $p < 0.05$) (Fig. 6). The decreased affinity of the regulatory domain of PKC for [^{3}H]PDBu after β-amyloid treatment was correlated with a significant increase in the total number of binding sites

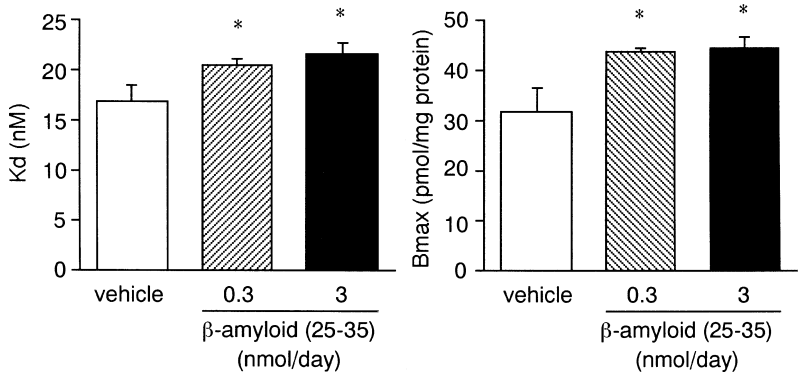

Fig. 6. The dissociations constant (kD) and the total number of binding sites (Bmax) resulted from the Scathard plot of the [3H] PDBu binding in the hippocampal particulate fraction on day 18 after the treatment with β-amyloid (25–35). Columns denote the means ± SEM of 6 experiments done in duplicate. *$p < 0.05$ vs. vehicle

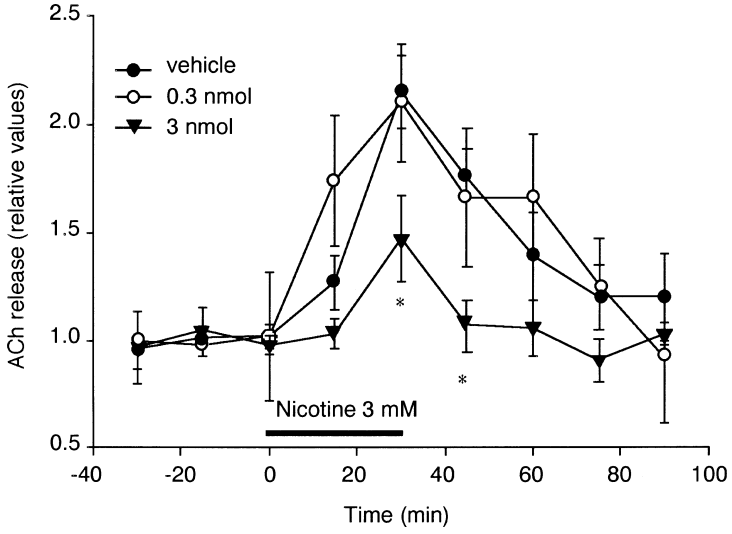

Fig. 7. The extracellular nicotine-evoked ACh release measured by microdialysis in the frontal cortex/hippocampus on day 10 after the start of β-amyloid (25–35) infusion. The values represent the mean ± SEM. The number of the measurements in each group were for vehicle = 5, for 0.3 nmol/day β-amyloid = 6, and for 3 nmol/day β-amyloid = 18. *$p < 0.05$ vs. vehicle

detected in the particulate fractions in the hippocampus (43.74 ± 0.88 and 44.36 ± 2.37 pmol/mg protein for 0.3 and 3 nmol/day β-amyloid treated groups, respectively, when compared with 31.72 ± 4.66 pmol/mg protein in the vehicle group; $p < 0.05$).

Effect of β-amyloid (25–35) on ACh release

As shown in Fig. 7, administration of β-amyloid fragment at the concentration of 3 nmol/day for 10 days into cerebral ventricle showed a significant decrease in the nicotine-stimulated ACh release as compared with rat receiving vehicle (147 ± 20% of the basal level for 3 nmol/day β-amyloid vs. 215 ± 17% of the basal level for vehicle; $p < 0.05$). Rat infused with 0.3 nmol/day β-amyloid did not show any alteration of nicotine-evoked ACh when compared with vehicle. There was no significant difference in basal extracellular ACh level among 3 groups (0.224 ± 0.06 pmol/60 μl/15 min in vehicle, 0.278 ± 0.03 pmol/60 μl/15 min in 0.3 nmol/day β-amyloid, 0.556 ± 0.117 pmol/60 μl/15 min in 3 nmol/day β-amyloid).

Discussion

The cognitive deficits of β-amyloid (1–40) and (1–42) have been reported by our group (Yamada and Nabeshima, 2000). We showed that the infusion of the full-length peptide (1–40) or (1–42) at a concentration of 0.3 nmol/day into rat brain impairs both the short- and long-term memory (Nitta et al., 1994, 1997; Yamada et al., 1998, 1999a,b). The core fragment of β-amyloid (25–35) can mimic in vitro the neurotoxic effects of β-amyloid (1–40) (Yankner et al.,

1990). In the present study we showed that β-amyloid (25–35) chronically administered at the same concentration (0.3 nmol/day) did not significantly affect learning and memory. However, when the peptide was infused at a concentration 3 nmol/day, a marked impairment in both short- and long-term memory was achieved in these animals, suggesting that β-amyloid (25–35) is one order less potent than β-amyloid (1–40) and (1–42) to induce neurotoxicity in vivo. The present findings are supported by the molecular changes in the expression of brain-derived neurotrophic factor (BDNF) mRNA in the hippocampus. Although chronic infusion of neurotoxic β-amyloid peptides, including β-amyloid (1–40), (1–42) and (25–35), significantly, but temporally (days 3 to 7 after the start of β-amyloid infusion), induced BDNF mRNA expression in the hippocampus, the magnitude of the response induced by β-amyloid (25–35) was considerably less than those obtained by the β-amyloid (1–40) and (1–42) at the same concentration (Tang et al., 2000).

The spontaneous alternation may have a component of spatial working memory since the animals should recall the previous memory of explored arm in order to explore another arm in consecutive choices. Used as an index of spatial short-term memory we found that the spontaneous alternation behavior in the Y-maze task was significantly decreased in animals treated with β-amyloid (25–35). It seems unlikely that the decreased alternation was due to a reduced locomotor activity in the Y-maze since the total number of arm entries did not significantly differ among groups treated with β-amyloid or vehicle. Furthermore, to exclude a possibility of motor deficit induced by the amyloid protein, we recorded the locomotor activity of these animals in a novel environment such as the locomotor cage. β-Amyloid-treated rats at both concentrations displayed the same exploratory behavior in a new environment, as did the vehicle-treated animals. The results indicates that the reduced alternation in the Y-maze test after treatment with 3 nmol/day β-amyloid is due to the disruption of short-term memory, but not due to a deficit in locomotion.

The long-term memory was investigated by using the Morris water maze and multiple passive avoidance tests. In the probe test of water maze task, the animals treated with both concentrations of β-amyloid (25–35) spent less time in the platform quadrant than the vehicle-treated rats, although there was no significant difference in performance during the course of the initial training trials among three groups. Moreover, in the reversal learning test, in which the position of the platform was moved to the opposite quadrant from the initial training trials, the rats treated with the high concentration of β-amyloid (3 nmol/day) showed a marked impairment of learning as indicated by a significant increase in escape latency in the second trial. The memory impairment was not due to an impairment of swimming ability, since in the first trial of the initial and the reversal learning tests, the pattern and the latency to search the hidden platform did not differ between β-amyloid- and vehicle-infused rats. Collectively it is suggested that β-amyloid at a concentration of 3 nmol/day disrupts spatial memory. The results of the water maze test were confirmed by the multiple passive avoidance task. The concentration of 3 nmol/day β-amyloid induced a significant reduction of the step-through

latency in the retention test, suggesting also a deficiency in the long-term memory to remembering the experience of the electric footshock.

Having the above results we showed that the treatment with β-amyloid (25–35) at a concentration of 3 nmol/day impairs both the short- and long-term memory. Thus, it is suggested that memory impairment in our rat model depends on the amount of β-amyloid infused into the brain as well as its intrinsic neurotoxic effect. In transgenic mouse models for AD, on the other hand, cognitive impairment may be dependent on the gene expression levels, age and gender (Yu and Oberto, 2000; Yamada and Nabeshima, 2000).

Beside the memory deficits, the neuropsychiatric abnormalities are clinical characteristics of the AD patients (Weiner et al., 1997; Harwood et al., 1998, 2000). Although the clinical reports described an increased status of anxiety associated with the Alzheimer's dementia, the mechanism responsible for the anxiogenic symptom is not known (Harwood et al., 2000). There are limited numbers of investigations for the role of amyloidogenesis in the emotional changes of AD, and so far there are no available evidences for the effects of the short fragment (25–35) of β-amyloid on anxiety, although the fragment is known to be neurotoxic when infused in rodents brain (Harkany et al., 1999). In the present study we have administered β-amyloid (25–35) either at pico- or nanomolar concentrations in order to correlate the potential anxiogenic effects with the memory deficits and acetylcholine release. We have found that β-amyloid (25–35) at the concentration of 3 nmol/day significantly decreased the time spent in the open arms of the elevated plus maze, suggesting an increased level of anxiety in those animals, while the 0.3 nmol/day concentration of the amyloid did not disrupt the emotional behavior. Although it remains to be determined whether these behavioral alterations can be ameliorated by treatment with anxiolytic drugs, our results demonstrate for the first time an anxiogenic effect induced by β-amyloid in an animal model of AD.

Compelling clinical and experimental evidences demonstrated that the cholinergic deficits contribute to the cognitive impairment in AD (Coyle et al., 1983; Davies et al., 1976; Whitehouse et al., 1982). We have also previously demonstrated that 0.3 nmol/day β-amyloid (1–40) induced a decrease in nicotine-evoked ACh release in the frontal cortex/hippocampus, which may contribute to the memory deficit in β-amyloid infused rats (Itoh et al., 1996). Nonetheless there is no direct evidence in vivo that a reduction in ACh release could underlie the memory deficits of β-amyloid fragment (25–35). In the present study we clearly demonstrated that when injected into the rat brain at a concentration of 3 nmol/day, β-amyloid (25–35) significantly decreased the nicotine-evoked ACh release in the frontal cortex/hippocampus, while 0.3 nmol/day β-amyloid or vehicle did not have any effect. Vehicle itself did not modify the amplitude of the ACh release when compared with the naive rats (data not shown). The reduction of ACh release correlates well with the amnesic effects and increased emotionality and are in line with the recent clinical reports which suggested that some neuropsychiatric changes associated with AD are related to the cholinergic deficits in brains of AD patients (Levy et al., 1999). This fact indicates that the anxiogenic effect of chronic

infusion of (25–35) fragment of β-amyloid may also be related to the decreased ACh release in the frontal cortex/hippocampus.

To investigate the intracellular mechanism for the impairment of ACh release, we measured the PDBu binding as an index of activated PKC in the hippocampus. It is well known that the neurotransmitter release is coupled to the magnitude of Ca^{2+} influx through the presynaptic voltage-activated channels (Smith and Augustine, 1988) and that the PKC is involved in the neurotransmitter release from nerve terminals of the hippocampal slice (Parfitt and Madison, 1993; Diaz-Guerra et al., 1988; Shu and Selmanoff, 1988; Herrero et al., 1992; Coffey et al., 1993). On the other hand, it has been proposed that β-amyloid neurotoxicity is caused by a lowered threshold of neurons for injury (such as excitotoxicity, hypoglycemia or peroxidative stress) and by increasing the level of intracellular free Ca^{2+} (Mattson et al., 1992). One of the consequences of the increased intracellular Ca^{2+} level is the activation of PKC.

In the present study we showed that the treatment with β-amyloid (25–35) at both concentrations significantly decreased the affinity of PDBu binding and increased the total number of binding sites in the hippocampal membrane fractions. Measurement of PDBu binding was performed on day 18 after the start of β-amyloid infusion. Although the nicotine-evoked ACh release was assayed on day 10 of β-amyloid treatment when the behavioral deficits were detected, the observed alterations in PDBu binding could contribute to the reduced ACh release. It is possible that in the early stage of β-amyloid peptide infusion, a low level of β-amyloid may induce an activation of PKC, which followed by the translocation of the enzyme from the cytosol to the membrane. Although there is no direct evidence to support such idea, the in vitro studies that at the physiological level β-amyloid stimulates PKC sustain our hypothesis upon the stimulation of PKC (Luo et al., 1997). Due to the continuous infusion of the peptide into the brain, an increased concentration of the β-amyloid could alter the phosphoinositide metabolism of the membranes, leading to the disorganization of the biochemical structures, and thus reducing the binding affinity of PKC for diacylglycerol, a physiological activator of the enzyme. We consider that the altered PDBu binding may contribute to the impairment of ACh release as well as behavioral deficits. It is of interest to note that PKC is involved in the non-amyloidogenic cleavage of β-amyloid precursor protein (APP) (Nitsch et al., 1992; Jolly-Tornetta et al., 1998). Accordingly, our findings suggest that accumulation of neurotoxic levels of β-amyloid in the brain may alter PKC activity, and thereby disrupt non-amyloidogenic processing of APP.

In conclusion, in the present study we found that the neurotoxic potency of β-amyloid (25–35) in vivo to induce memory deficits is less compared with that of β-amyloid (1–40) and (1–42), and is correlated with a reduction of ACh release in the hippocampus. We also described, for the first time, an increased emotional behavior induced by nanomolar concentration of β-amyloid (25–35). As a possible cellular mechanism, altered PKC system could be involved in the memory deficits and behavioral changes in our animal model.

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