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Difference in toxicity of β-amyloid peptide with aging in relation to nerve growth factor content in rat brain

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Summary. Amyloid β-peptide (Aβ) is the major constituent of the senile plaques in the brains of patients with Alzheimer's disease. We have demonstrated previously that memory impairment, dysfunction of the cholinergic and dopaminergic neuronal system and morphological degeneration are produced after the continuous infusion of Aβ into the cerebral ventricle in 8-week-old rat. In the present study, we investigated the toxicity of Aβ in infant (10 days old), adult (8 weeks old) and aged (20 months old) rats in relation to nerve growth factor (NGF) content in various regions of the brain. After a 2-week-infusion, choline acetyltransferase (ChAT) activity was significantly decreased in the hippocampus of adult, but not infant or aged rats. NGF levels in the hippocampus were increased only in adult rats. These results suggest that Aβ is toxic only in the matured adult brain, and that the mechanism of toxicity is related to NGF synthesis.

Keywords: Amyloid β-peptide, nerve growth factor, choline acetyltransferase, Alzheimer's disease, enzyme immunoassay, HPLC-ECD method, rats.

Introduction

Alzheimer's disease (AD) is characterized by the presence of senile plaques and neurofibrillary tangles accompanied by synaptic and neuronal loss (Maters et al., 1985). The core of the plaque consists of amyloid β-peptide (Aβ) and other proteins. The extent of Aβ deposition correlates with the degree of neuronal damage, cognitive impairment and memory loss (Wilcock et al., 1982). We have demonstrated previously that memory impairment, dysfunction of the cholinergic neuronal system and morphological degeneration are produced after the continuous infusion of Aβ (1-40) into the cerebral ventricle in 8-week-old rats (Nabeshima and Nitta, 1994; Nitta et al.,

1997, 1994). Furthermore, nicotine- and K^+-stimulated acetylcholine and/or dopamine release from the frontal cortex/hippocampus and striatum, respectively, was impaired in Aβ-infused rats (Itoh et al., 1996). In the study of neural activity, nicotine reduces the amplitude of electrically evoked population spikes in the CA1 pyramidal cells of the vehicle control rats, but not in those of the Aβ-infused rats (Itoh et al., 1999). Long-term potentiation induced by tetanic stimulations in CA1 pyramidal cells, which was readily observed in the vehicle control rats, was also impaired in the Aβ-infused rats (Itoh et al., 1999). Nerve growth factor (NGF) is a main member of the neurotrophin family of proteins, which influence the survival and/or differentiation of various types of central neurons. NGF has been reported to stimulate cholinergic function (Mobley et al., 1985), and to prevent the death of cholinergic medial septum cells following axotomy (Williams et al., 1986). Moreover, NGF has been shown to enhance learning behavior in aging rats, while infusion of an anti-NGF antibody into the septum impairs learning ability and cholinergic neuronal ability (Fischer et al., 1987; Nitta et al., 1993). Further, peripheral administration of idebenone or propentofylline, which stimulate NGF synthesis in cultured mouse astrocytes and in the brains of aged rats, improves learning and memory deficits in rats induced by Aβ (Nabeshima et al., 1993; Nitta et al., 1993; Shinoda et al., 1990; Takeuchi et al., 1990; Yamada et al., 1998, 1999). These results suggest that NGF itself and/or NGF-related compounds could be useful for the treatment of AD. In this study, we investigated the toxicity of Aβ in infant, adult and aged rats in relation to NGF content, since the role of NGF in the Aβ toxicity in brain has yet to be elucidated.

Materials and methods

Animals

Male Kbl Wistar rats at the age of 10-days (infant), 8 weeks (adult) and 20 months (aged)(Oriental Bio Service, Co., Kyoto) (Nitta et al., 1994) 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 animals were handled in accordance with the guidelines established by the Institute for Laboratory Animals Research of this university. The rats had free access to food and water.

Infusion of Aβ

The synthesized human Aβ (1-40) was obtained from Bachem (Torrance, CA, USA). The Aβ was dissolved in 35% acetonitrile/0.1% trifluoro acetic acid (TFA). Continuous infusion of the Aβ (300pmol/day; most toxic dose) was maintained for two weeks by attaching a cannula to a modified mini-osmotic pump (Alzet 2002; Alza, Palo Alto, CA, USA) (Nitta et al., 1994, 1997). The control rats were only infused with the vehicle (35% acetonitrile/0.1% TFA), because there are no differences in toxicity between vehicle and Aβ (40-1) as shown in a previous report (Nitta et al., 1997). The cannula was implanted into the right ventricle (A −0.3, L 1.1, V 3.6). After a 2-week infusion, rats were decapitated, and various regions of the brain measured for ChAT activity and NGF content by high-performance-liquid chromatography with an electron capture detector (HPLC-ECD) and by enzyme immunoassay (EIA), respectively (Nitta et al., 1993).

NGF assay

NGF content was measured by enzyme immunoassay (EIA) (Nitta et al., 1993).

U-bottomed 96-well immunoplates (Falcon 3910)were coated with 10μl of anti-mouse NGF monoclonal antibody (Boehringer Mannheim Biochemicals, 1μg/ml in 50mM sodium carbonate buffer, pH 9.6) per well for 2hr at room temperature. After 3 washes with 150μl of washing buffer (0.1 M Tris-HCl buffer containing 0.4 M NaCl, 0.02% Na_3N, 0.1% BSA, and 1 mM $MgCl_2$), the unoccupied space on the surface of the plate was blocked by rinsing with 150μl of BSA in the washing buffer for 1hr at room temperature. The BSA solution was then removed. Twenty-μl samples or NGF standard solution (Boehringer Mannheim Biochemicals, 0.1 U/ml in washing buffer) was added to the wells (30μl) and the plate was sealed and incubated overnight at 4°C with gentle shaking, before being washed three times with 150μl of the washing buffer. Substrate for the enzyme (Boehringer Mannheim Biochemicals; 30μl of 4-methylumbelliferyl-β-D-galactoside; 20μg/ml washing buffer) was then added to the plate, which was then sealed and incubated for 5hr at room temperature. The enzyme reaction was stopped by the addition of 100μl of 0.1 M glycine-NaOH buffer (pH 10.3), following which the reaction mixture was transferred to new tubes containing 2.0ml of the same buffer for the determination of fluorescence intensity. This was performed with a fluorospectrophotometer (Shimadzu, RF-540, Kyoto, Japan), with wavelengths for excitation and emission being 360nm and 450nm, respectively.

Measurement of ChAT activity

ChAT activity was determined as previously reported (Nitta et al., 1993). Each brain tissue was sonicated for 5s in 12.5ml of cold 0.025M phosphate buffer, pH 7.4, containing 0.5% triton X-100, per g of wet weight. The homogenate was centrifuged at 20,000× g for 30min. ChAT activity in the supernatant was assayed. The sample was stored −80°C until used.

In the incubation, 100μl of substrate solution containing 10mM choline chloride, 0.4mM acetyl Co-A, 0.2mM eserine sulfate, 0.3M sodium chloride and 20mM EDTA-2Na in 0.1M sodium phosphate buffer, pH 7.4, was added to 100μM of enzyme solution in 25mM sodium phosphate buffer, pH 7.4, and was incubated at 37°C for 20min. The enzyme reaction was stopped with 50μl of 1M perhydrochloride in ice. After 10min, 6μl of 1.0mM isopropylhomocholine was added as an internal standard and the mixture was centrifuged at 16,000× g for 10min at 4°C. A 150μl aliquot of the clean supernatant was taken and passed through Millipore filter (0.45μm). A 10μl aliquot was injected into the HPLC system. The HPLC system comprised a column (styrene polymer, Eicom AC-Gel, 6 × 150mm) and an enzymatic post column reactor containing acetylcholinesterase and choline oxidase, convalently bound to amino propyl silica gel. Acetylcholine and choline were converted to hydrogen peroxide, which was then detected electrochemically (Eicom ECD-100, Kyoto) with a platinum electrode at 450mV. The column temperature was 33°C. The mobile phase was delivered with a single piston pump (Shimadzu) at 1.0ml/min, and was 0.1M phosphate buffer at pH 8.5, containing 0.6mM tetrametyl ammonium chloride (Nacalai Tesque) and 1.2mM sodium 1-decanesulfonate (Tokyo Kasei).

Results

ChAT activity

In the vehicle infused control rats, no differences were observed in ChAT activities in any regions of the brain among infant, adult or aged rats (Table 1 and Fig. 1). As shown in Fig. 1, ChAT activity in the hippocampus was reduced by the infusion of Aβ only in the adult, not in the infant or aged rats. Aβ failed to reduce ChAT activity in any other regions. These results from

Table 1. Effects of continuous infusion of β-amyloid protein on ChAT activity and NGF content in various brain regions in infant, adult and aged rats

Brain region	Infant		Adult		Aged	
	vehicle	β-amyloid	vehicle	β-amyloid	vehicle	β-amyloid
ChAT activity (pmol/min/mg)						
frontal cortex	47.1 ± 4.58	49.2 ± 3.9	41.5 ± 6.7	50.3 ± 6.4	51.1 ± 3.5	49.6 ± 2.7
parietal cortex	93.9 ± 3.62	105.1 ± 9.4	96.5 ± 3.1	85.5 ± 3.8	91.1 ± 7.8	92.8 ± 7.9
striatum	514.6 ± 33.3	481.1 ± 55.4	447.5 ± 38.8	396.5 ± 28.7	390.45 ± 16.8	393.2 ± 45.8
NGF contents (pg/g)						
frontal cortex	1,761.3 ± 237.1	1,892.5 ± 607.1	1,888.3 ± 332.1	1,853.2 ± 230.2	1,858.2 ± 488.8	1,850.1 ± 110.1
parietal cortex	1,672.5 ± 149.5	1,640.1 ± 83.4	1,922.4 ± 116.4	1,866.2 ± 180.2	1,762.5 ± 135.2	1,822.5 ± 149.51
striatum	822.8 ± 155.5	719.2 ± 296.1	858.6 ± 196.7	900.3 ± 250.6	693.3 ± 165.6	900.3 ± 250.6

Each value shows the mean ± SEM of 5 animals

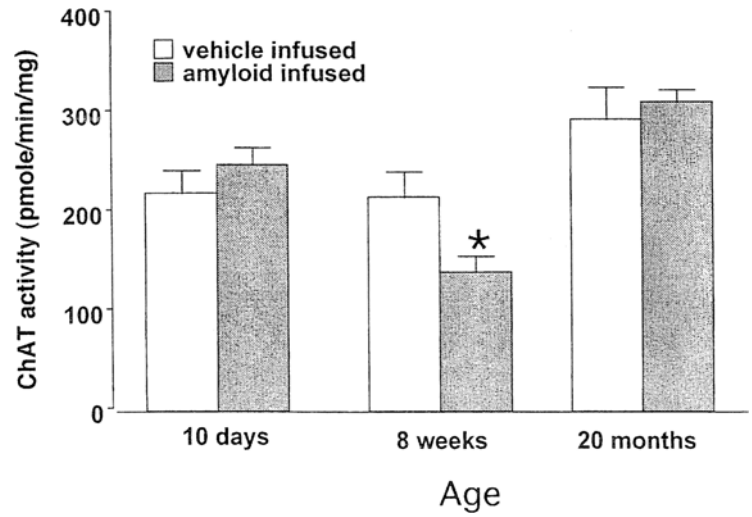

Fig. 1. Effects of continuous infusion of β-amyloid protein on ChAT activity in the hippocampus in infant (10-day old), adult (8-week old) and aged (20-month old) rats. Each value shows the mean ± S.E.M. of five animals. *$p < 0.05$ vs. vehicle-treated rats (Tukey's test)

adult rats reconfirmed those of our previous reports (Nabeshima and Nitta, 1994; Nitta et al., 1993, 1997). In these reports, ChAT activity in the cortex was reduced by the infusion of Aβ. The discrepancies in results are considered to be due to differences in the manner in which the brain was dissected. However, ChAT activity in the infant or aged rats was not changed by the infusion of Aβ.

NGF content

There were no age differences in the NGF content of the various brain regions of control animals (Fig. 2 and Table 1). However, as shown in Fig. 2, NGF levels in the hippocampus increased in adult rats after the infusion of Aβ. Aβ did not increase NGF levels in any other region. The NGF content of the hippocampus was not changed in the infant or aged rats.

Discussion

In the present study, ChAT activity was not reduced with aging, except in the striatum. We found that the activity of ChAT, a marker enzyme in cholinergic neurons, did not change with age. Previous reports have shown that ChAT activity is reduced with age in a number or few regions of the brain (Ishimaru et al., 1991; Luine and Hearns, 1990; Nabeshima et al., 1993; Nitta et al., 1993). These discrepancies are considered to be due to differences in the animal strains used, or in the environment in which the rats were reared.

The most important findings in this study are that infusion of Aβ induces cholinergic neuronal degeneration only in matured brain, not in aged or unmatured ones. And only in adult brain was NGF content increased in the

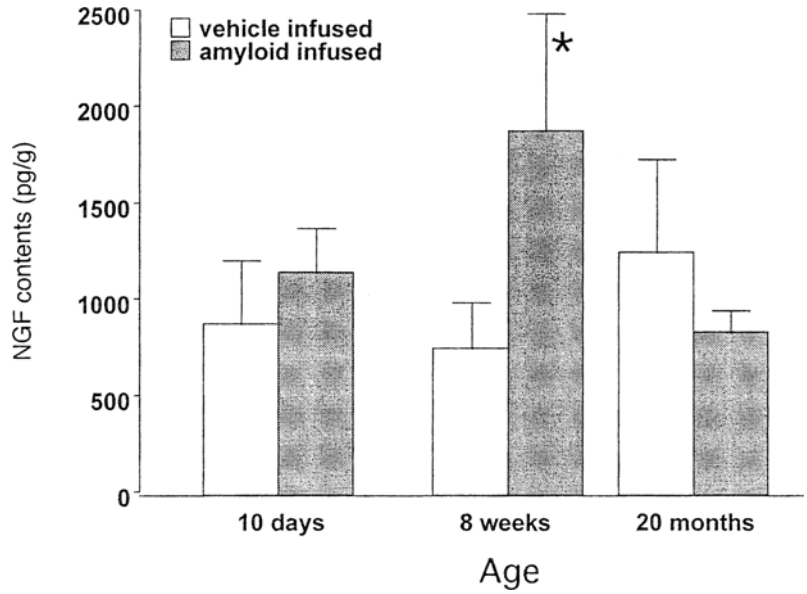

Fig. 2. Effects of continuous infusion of β-amyloid protein on NGF content in the hippocampus in infant (10-day old), Adult (8-week old) and aged (20-month old) rats. Each value shows the mean ± S.E.M. of five animals. *$p < 0.05$ vs. vehicle-treated rats (Tukey's test)

hippocampus. These results suggest that the toxicity of Aβ is related to NGF production only in matured brains. The mechanism of regulatory system of NGF production is not clarify completely. When a long-lasting enhancement of c-jun mRNA expression is increased, NGF production is induced (Omae et al., 1994). c-jun generates activator protein-1 that drives NGF gene expression (Hengerer et al., 1990). After the infusion of Aβ, expression of immuno-reactivity of c-fos was increased (Nitta and Nabeshima, unpublished data). c-fos is always bind to c-jun and then activates gene transcritions, including NGF gene (Hengere et al., 1990).

It has been reported by Eriksdotter-Jönhagen et al. (1998) that infusion of NGF into the brains of patients with AD resulted in an increase of nicotine-binding in the frontal cortex and temporal cortex. This case report suggests that NGF counteracts the cholinergic deficits in senile AD. However, the relation between AD and NGF has yet to be clarified. Several studies (Ernfors et al., 1990; Fahnestock et al., 1996; Murase et al., 1993; Scott et al., 1995) have failed to corroborate the hypothesis of a decreased NGF synthesis in the brain, either in Alzheimer patients or in most aged rodents; rather, they indicated an up-regulation of the NGF system. According to various post-mortem studies of AD brains, NGF mRNA expression in the cortex and hippocampus is unchanged (Ernfors et al., 1990). Moreover, marked increases in NGF content in several cortical and subcortical regions, have been found (Fahnestock et al., 1996). In contrast to target regions of the cholinergic basal forebrain, the nucleus basalis of Meynert of AD patients was reported to have low NGF levels (Goedert et al., 1986). This could indicate a defective

retrograde transport of target-derived NGF with subsequent elevation of NGF protein in target regions. In fact, recent immunocytochemical and in situ hybridization experiments have shown that the expression of the high-affinity NGF receptor trkA is significantly reduced in AD neurons of NBM (Hock et al., 1998; Mufson et al., 1996, 1997; Salehi et al., 1996), parietal cortex and striatum, while normal mRNA levels for the low-affinity neurotrophin receptor (p75) and the remaining high-affinity neurotrophin receptor (trkB and trkC) were found in surviving cholinergic neurons of the parietal cortex and NBM (Boissiere et al., 1997). In human brain, the most severe deposits of Aβ were observed in the cerebral cortex, in which NGF levels are increased as described above (Mufson et al., 1996; Narisawa-Saito et al., 1996; Nyberg and Waller, 1989). In our AD model rats, an increase in the NGF content of the hippocampus was found, although this is one of target regions in the cholinergic neuronal system. Aβ was infused into cerebral ventricle in our AD model. The hippocampus was exposed to the cerebral ventricle directly, and deposit of Aβ were found there (Nitta et al., 1993). The condition in the hippocampus in our AD model may be similar to those the cortex in AD human.

Very little is known about the pathogenic relationship between changes in NGF content and Aβ toxicity in AD brain. Hellweg et al. (1998) have obtained interesting results about the relationship among dementia scale, NGF content and Aβ. In the AD group, the NGF content of frontal cortex and temporal cortex was increased as compared with in the non-dementia group. In a subgroup analysis of non-dementia patients, NGF levels were lower in the frontal cortex when Aβ plaques were present than absent. The decrease of NGF content paralleled the decrease of ChAT activity. These results support the hypothesis of a reduced cortical NGF content at the onset of plaque formation and of elevated NGF levels in the clinically manifest and neuropathologically advanced stage of the disease.

In the adult brain, NGF content increased with the toxicity of Aβ, however the degree of protection was not high enough to maintain a normal level of ChAT activity. In the brain in the AD model (8 week-old), immunoreactivity for Aβ was observed, but senile plaques were not (Ikeda, K., Nitta, A. and Nabeshima, T., unpublished data). The NGF induced by Aβ injection into adult brain might prevent formation of senile plaques. In infant and aged brain, the NGF regulatory system(s) and ChAT activity did not respond to Aβ infusion. The difference of Aβ toxicity could be because the nervous functions are not fully developed. Notably, blood-brain barrier (BBB) of the infant brain has not been established while that of the aged brain is no longer fully functional (Conford and Conford, 1986). Therefore some proteins and/or peptides could enter the brain from peripheral blood, and attenuate the toxicity of Aβ. In the recent report has demonstrated that peripherally administered antibodies against Aβ-peptide enter the central nervous system and reduce pathology in Alzheimer' s disease (Iwata et al., 2000). Further, major Aβ-degrading and catabolic pathway are due to the BBB function. These results suggest that the disorder of BBB may cause Alzheimer's disease (Bard et al., 2000). In fact, It is reported that the BBB is

partially destroyed in some neurological disorders such as multiple sclerosis and Alzheimer's disease (Elovaara et al., 1985; Conford and Conford, 1986). In addition, it may be hard to deposit Aβ in infant and aged brains, because it easily passes through the BBB. Further studies should be done to clarify the system of protection against Aβ infusion.

In conclusion, the present results demonstrate that Aβ is toxic only in the matured adult brain, and that the mechanism of toxicity may be related to NGF content.

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