ELSEVIER

European Journal of Pharmacology 382 (1999) 167–175

ejp

www.elsevier.nl/locate/ejphar

Impairments of long-term potentiation in hippocampal slices of β-amyloid-infused rats

Akio Itoh [a], Tadashi Akaike [b,c], Masahiro Sokabe [b], Atsumi Nitta [d], Ryuichi Iida [a], Ana Olariu [a], Kiyofumi Yamada [a], Toshitaka Nabeshima [a,*]

[a] *Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8560, Japan*

[b] *Department of Physiology, Nagoya University School of Medicine, Nagoya 466-8560, Japan*

[c] *Department of Oral Physiology, Hokkaido University School of Dentistry, Sapporo 060-0811, Japan*

[d] *Department of Molecular Biology, Gifu Pharmaceutical University, Gifu 502-0003, Japan*

Received 1 April 1999; received in revised form 12 August 1999; accepted 13 August 1999

Abstract

In this study, we investigated the neuronal activity of hippocampal slices from the β-amyloid protein-infused (300 pmol/day for 10–11 days) rats using the extracellular recording technique. Perfusion of nicotine (50 μM) reduced the amplitude of electrically evoked population spikes in the CA1 pyramidal cells of the vehicle control rats, but not in those of the β-amyloid protein-infused rats, suggesting the impairment of nicotinic signaling in the β-amyloid protein-infused rats. 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 β-amyloid protein-infused rats. Nicotinic blockade by adding hexamethonium into the perfused solution inhibited long-term potentiation induction. Taken together, our previous and present results suggest that β-amyloid protein infusion impairs the signal transduction mechanisms via nicotinic acetylcholine receptors. This dysfunction may be responsible, at least in part, for the impairment of long-term potentiation induction and may lead to learning deficits.

Keywords: Alzheimer's disease; β-amyloid protein; Hippocampus; Nicotine; Learning; Memory; Long-term potentiation

1. 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). The central cholinergic neuronal system plays an important role in learning and memory (Nabeshima, 1993), therefore, 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).

The core of SP consists of β-amyloid protein (Aβ), a polypeptide of 39–43 amino acids, which is a product of the proteolytic cleavage of β-amyloid protein precursor (APP) (Haas et al., 1992; Shoji et al., 1992; Selkoe, 1994). Several genetic and transgenic studies have indicated that excess amount of Aβ is produced by mutations of 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., 1990). One possible mechanism of Aβ neurotoxicity is the disruption of intracellular Ca^{2+} homeostasis (Mattson et al., 1993) by impairing function of ion-regulating protein such as K^+ channel (Etcheberrigaray et al., 1993, 1994) and ion transporters (i.e., Na^+/K^+-ATPase and Ca^{2+}-ATPase) (Mark et al., 1995). Moreover, Arispe et al. (1993a,b) have reported Aβ can form Ca^{2+} channels in

* Corresponding author. Tel.: +81-52-744-2674; fax: +81-52-744-2682; e-mail: tnabeshi@tsuru.med.nagoya-u.ac.jp

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00601-9

planar lipid bilayer membranes. However, the details of Aβ toxicity remain to be elucidated.

So far only a few acetylcholinesterase inhibitors such as tacrine and donepezil have been developed as effective therapeutic drugs for AD. This is due to the lack of the suitable animal models of AD. To date, electrical or chemical lesions of cholinergic neurons in the basal forebrain have been employed to produce animal models of AD (Nabeshima, 1993). These models show AD-like cognitive dysfunction, however, they are not suitable for investigating causes and development of AD. It is necessary to develop an adequate animal model of AD. Although APP transgenic mice are useful models of AD with which to elucidate the process of Aβ deposition and neuronal degeneration, it takes for about 1 year to develop the learning and memory deficits in these mutant mice (Hsiao et al., 1996; Nalbantoglu et al., 1997).

We have reported that continuous (2 weeks) infusion of Aβ (1–40, 300 pmol day^{-1}) into the cerebral ventricle of rats induced learning deficits, and decreases choline acetyltransferase (ChAT) activity in the frontal cortex and hippocampus (Nabeshima and Nitta, 1994; Nitta et al., 1994, 1997). Immunohistochemical analysis revealed diffuse depositions of Aβ in the cerebral cortex and hippocampus around the ventricle and persistent activation of astroglial cells (Nabeshima and Nitta, 1994; Nitta et al., 1994, 1997). Furthermore, the nicotine-stimulated release of acetylcholine and dopamine in the frontal cortex/hippocampus and striatum, respectively, is decreased in the Aβ-infused rats (Itoh et al., 1996). These results suggest that signal transduction via nicotinic receptors is impaired by the continuous infusion of Aβ and that the dysfunction of neurotransmitter releases may be one of the causes of the learning deficits observed in these animals.

In this study, therefore, we investigated whether Aβ-infusion changes nicotinic responses in hippocampal CA1 pyramidal cells in brain slices using the extracellular recording technique. In addition, we investigated the effect of Aβ-infusion on long-term potentiation (LTP) in these cells.

2. Materials and methods

2.1. Animals and chemicals

Male Kbl Wistar rats (Oriental Bio Service, Kyoto, Japan), weighing 280–300 g (7 weeks old) at the beginning of the experiments, were used. Aβ (1–40) was purchased from Bachem (Torrance, CA) and nicotine, mecamylamine and hexamethonium were purchased from Sigma (St. Louis, MO), respectively. Other chemicals (special grade) were obtained from Wako (Osaka). Nicotine, mecamylamine and hexamethonium were dissolved with standard (STD) solution (in mM: NaCl 128, KCl 1.7, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10).

2.2. Surgery

The animals were handled in accordance with the guidelines established by the Institute for Laboratory Animal Research of this university, and housed in a temperature- and light-controlled room (23°C, a 12-h cycle starting at 9:00 am). They had free access to food and water. The procedure for infusion of Aβ was described previously (Nabeshima and Nitta, 1994; Nitta et al., 1994, 1997). Aβ was dissolved in 35% acetonitrile/0.1% trifluoroacetic acid to prohibit aggregation of Aβ in the osmotic minipump. Continuous infusion of Aβ was maintained by the osmotic minipump (Alzet 2002; Alza, CA) with a cannula that was implanted into the ventricle (A-0.3; L 1.1; V 4.0 mm) according to the atlas of Paxinos and Watson (1986). In this study, only one dose of Aβ (300 pmol day^{-1}) was tested. At this dose, the protein induced severe learning deficits and reduction of ChAT activity (Nabeshima and Nitta, 1994; Nitta et al., 1994, 1997), and the dysfunction of neurotransmitter releases (Itoh et al., 1996). The vehicle control rats were infused vehicle only, because vehicle alone and reverse Aβ (40–1) showed no behavioral or biochemical changes (Nitta et al., 1997). The numbers of slices used in the experiments (each animal provided one or two slices) were expressed as '*n*' in the text and figure legends.

2.3. Preparation of hippocampal slices

Ten or eleven days after starting Aβ infusion, rats were sacrificed by decapitation and the brains were quickly removed and placed in ice cold STD solution (continuously gassed with 95% O_2 and 5% CO_2) for about 5 min. Slices (400 μm thick) were then cut in the coronal plane using a vibratome (Microslicer DTK1500, Dousaka EM, Kyoto, Japan). Slices were placed on small pieces of filter paper presoaked in STD solution at room temperature for more than 1 h to allow the recovery from the cutting damage. Then, they were transferred to a recording chamber fixed to an Olympus inverted microscope (IMT-2). The acryl resin chamber had an 18-mm hole, the bottom of which was covered with a cover-slip attached to the chamber with a thin layer of silicone. The hole was connected to inlet and outlet perfusion tubings made of Teflon®. The slices were perfused continuously with STD solution at a flow rate of about 2 ml min^{-1}.

2.4. Stimulation and recording

A slice was kept in the recording chamber, and two weights made of silver wire were placed on it to fix the slice on the cover-slip. Two or three hydraulic Narishige

micromanipulators were mounted on the same microscopic stage. They were used to place a bipolar metal electrode, insulated except at its tip, in the radiatum–lacunosum layers to stimulate Shaffer collaterals and/or commissural fibers in the CA1 region. A recording glass pipette filled with the STD solution (without glucose) and having 2–5 MΩ resistance was placed in the pyramidal cell layer near the stimulating electrode. The stimulation was controlled by a constant current stimulator (Nihon Khoden, Tokyo). The intensity of the test stimuli was adjusted to evoke about 50% of the maximum response. Stimuli were given every 30 s. The recording electrode was connected to a neutralized, high input-impedance preamplifier. Electrical signals were digitized at a bin width of 50–100 μs, monitored and saved with the pCLAMP system (Axon Instrument, CA). The effects of nicotine were determined by perfusing the STD solution gassed with 95% O_2 and 5% CO_2 containing nicotine (50 μM) for 10 min or 30 s and then normal STD solution gassed with 95% O_2 and 5% CO_2 was perfused. To induce LTP, a tetanic stimulation (100 Hz for 1 s, same intensity during the basal stimulation) was applied. In this study, we employed PS amplitude as synaptic activity. In the nicotinic blocking experiment, the nicotinic blocker hexamethonium (50 μM) was added into perfused STD solution. The control population spike (PS) amplitude was recorded 10 and 15 min before the application of nicotine and tetanic stimulation, respectively. The PS amplitude was evaluated by taking the voltage difference between the onset and peak of PS. All experiments were performed at the room temperature from the beginning to the end in the temperature-controlled room (25 ± 1°C).

2.5. Data analysis

The obtained PS amplitude was averaged and expressed as ''fold increase'' to its control level which was measured before application of nicotine and tetanic stimulation. Two-factor ANOVA was used to compare the differences of response among the groups. When the significant difference was observed, the values at the end of observation (45 min after tetanic stimulation) in LTP experiments and the mean values taken from 5 to 10 min after the start of nicotine application in nicotine experiments both values were compared by means of Scheffe's *F*-test.

3. Results

3.1. Effects of nicotine on PS amplitude in hippocampal CA1

To confirm our previous result (Itoh et al., 1996) that suggested the impairment of nicotinic signal transduction in the Aβ-infused rats, we compared the electrophysiological responses in hippocampal CA1 pyramidal cells of the vehicle control and Aβ-infused rats during nicotine application.

We could not find the significant differences on electrically evoked PS amplitude between vehicle control and

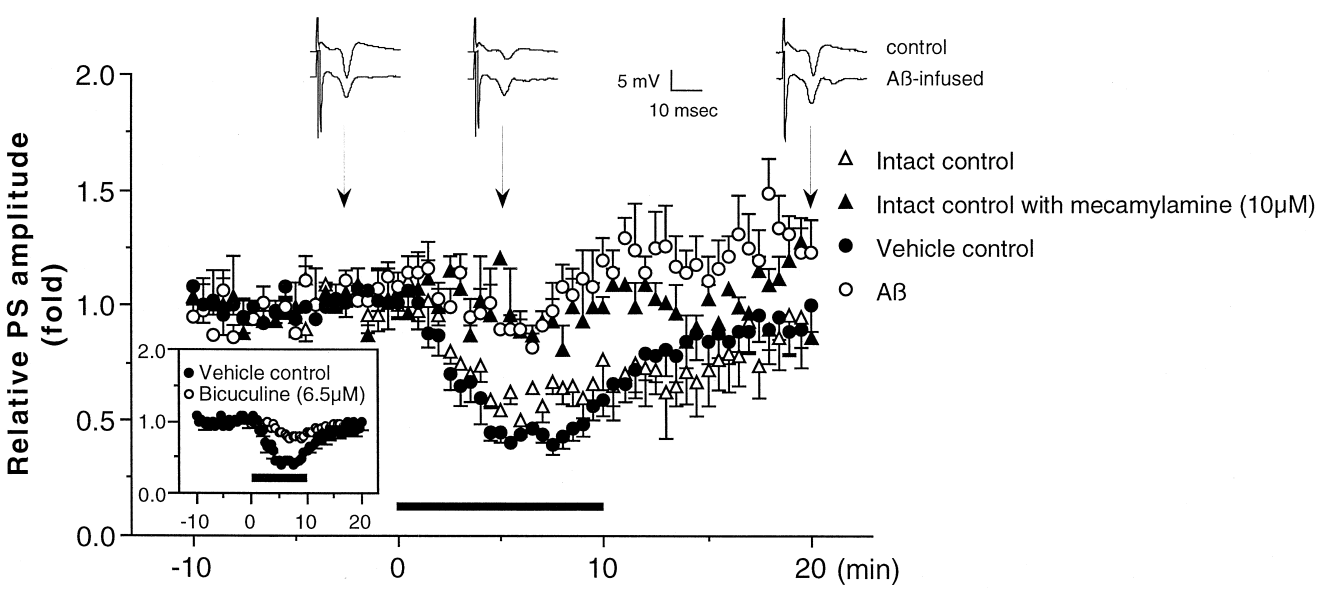

Fig. 1. Effects of nicotine (50 μM) on the PS amplitude in the hippocampal CA1 pyramidal cells of the intact control, vehicle control and Aβ-infused rats. A standard solution with nicotine was applied for 10 min as indicated by a thick bar and then changed to a standard solution without nicotine. In antagonist experiment, mecamylamine was perfused at the concentration of 10 μM throughout the experiment (inserted). Nicotine-induced decrease of PS amplitude was diminished by bicuculline (6.5 μM). Each value represents a relative amplitude to the control level (mean ± S.E.M.). $F_{1,483} = 282.422$, $P < 0.01$ (vehicle control vs. Aβ-infused rats). The numbers of slices and animals are as follows: intact control $n = 3$; vehicle control $n = 5$; Aβ $n = 5$; vehicle control with mecamylamine $n = 3$; intact control with bicuculline $n = 4$.

Aβ-infused rats (4.37 ± 1.01, 4.46 ± 0.67 mV, respectively) before drug treatment or tetanic stimulation. However, the current to obtain half maximal response in Aβ-infused rats tended to be higher than that in vehicle control rats (vehicle control; 0.54 ± 0.10, $n = 12$: Aβ; 0.86 ± 0.24 mA, $n = 13$).

Application of nicotine (50 μM) into perfused STD solution decreased the PS amplitude in hippocampal CA1 pyramidal cells of the vehicle control rats ($n = 5$: Fig. 1) as well as the intact (not treated) control that received no treatment ($n = 3$: Fig. 1). Five minutes after the application of nicotine, the PS amplitude decreased to approximately half the vehicle control level. The PS amplitude returned to the control level 5 min after perfusion with a STD solution without nicotine. There was no significant difference in nicotine-induced reduction of PS amplitude between the intact control and vehicle control rats. The nicotine-induced reduction of PS amplitude was attenuated by co-application of nicotinic antagonist mecamylamine (10 μM: $n = 3$: Fig. 1), indicating that the reduction was mediated via nicotinic acetylcholine receptors. Moreover, application of the γ-aminobutyric acid (GABA) antagonist bicuculine throughout the experiment also attenuated the reduction ($n = 4$: Fig. 1). In the Aβ-infused rats ($n = 5$) however, the degree of nicotine-induced reduction of PS amplitude was significantly less than that in the vehicle control rats ($F_{1,483} = 282.422$, $P < 0.01$: Fig. 1).

3.2. Induction of LTP in hippocampal CA1

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

Immediately after a tetanic stimulation, the PS amplitude was dramatically enhanced in the intact control ($n = 3$) and vehicle control ($n = 7$) rats to about four–five-fold of the control level (post-tetanic potentiation; Fig. 2). The PS amplitude gradually decreased to a level about two-fold that of the control level, indicating the induction of LTP in the vehicle control rats (Fig. 2). Although in the Aβ-infused rats ($n = 8$), a similar degree of enhancement of PS was observed immediately after tetanus, the PS amplitude soon returned to the control level. Although the PS amplitude of Aβ-infused rats after tetanic stimulation was slightly higher compared to that of their control level, it was significantly lower than that of the vehicle control rats ($F_{1,1417} = 833.538$, $P < 0.01$; Fig. 2) and the PS amplitude at the end of observation (45 min) of control rats was significantly higher than that of Aβ-infused rats ($P < 0.05$).

3.3. Effects of nicotinic blockade on LTP induction

Since we speculated that dysfunction of signal transduction of nicotinic acetylcholine receptor may be responsible for impairment of LTP induction in Aβ-infused rats, we investigated whether nicotinic blockade suppresses LTP induction. Although it has been known that hexamethonium is hardly delivered into the brain when administered systemically, it can block central nicotinic acetylcholine receptors when administered directly to brain slices. In this experiment, hexamethonium was used to block nicotinic acetylcholine receptors.

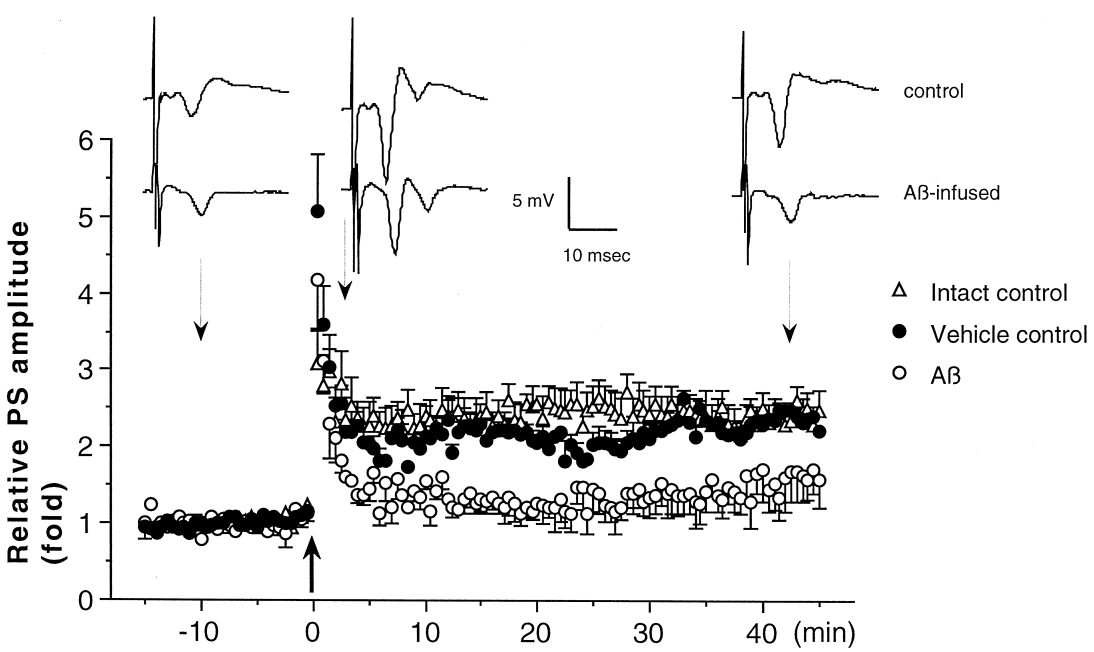

Fig. 2. Enhancement of PS amplitude after tetanic stimulation (100 Hz for 1 s) in the hippocampal CA1 pyramidal cells of the intact control, vehicle control and Aβ-infused rats. An arrow indicates the time when tetanic stimulation was applied. Each value represents a relative amplitude to the control level (mean ± S.E.M.). $F_{1,1417} = 833.538$, $P < 0.01$ (vehicle control vs. Aβ-infused rats). The numbers of slices and animals are as follows: intact control $n = 3$; vehicle control $n = 7$; Aβ $n = 8$.

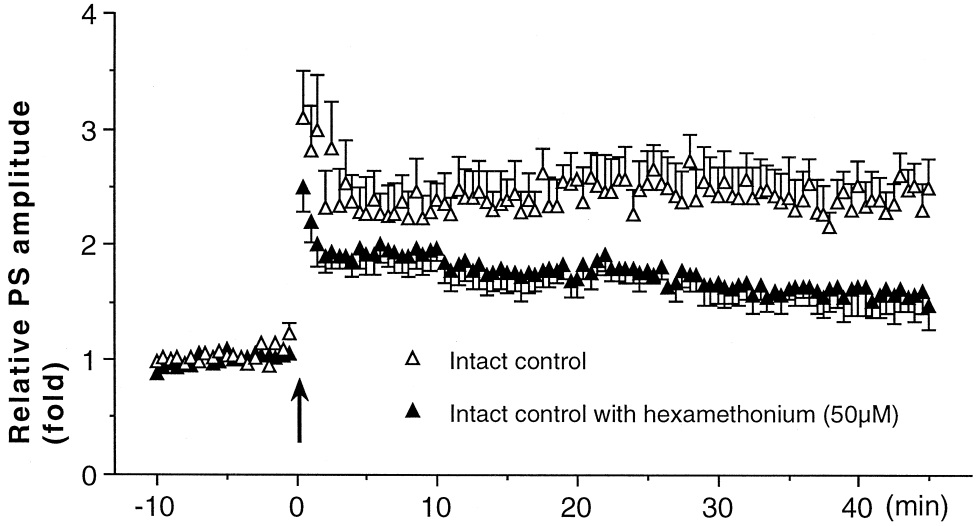

Fig. 3. Inhibition of LTP induction by application of the nicotinic blocker, hexamethonium (50 μM), in the hippocampal CA1 pyramidal cells of the control rats. An arrow indicates the time when tetanic stimulation was applied (100 Hz for 1 s). Hexamethonium was perfused throughout experiments. Each value represents a relative amplitude to the control level (mean ± S.E.M.). $F_{1,1042} = 431.925$, $P < 0.01$ (control vs. hexamethonium). The numbers of slices and animals are as follows: control $n = 7$; hexamethonium $n = 6$.

As shown in Fig. 3, LTP could be induced in the slices of intact control ($n = 7$) rats by tetanic stimulation, however, it was attenuated by the presence of hexamethonium (50 μM: $F_{1,1042} = 431.925$, $P < 0.01$: $n = 6$) throughout the experiment. The PS amplitude at the end of observation (45 min) was significantly higher in control slices compared to hexamethonium-perfused slices ($P < 0.05$).

3.4. Effects of nicotine on the impairment of LTP induction in Aβ-infused rats

The results described above suggested that dysfunction of nicotinic signal transduction was induced in Aβ-infused rats and that this dysfunction is responsible for the impairment of LTP induction. Next, we attempted to find out whether nicotine ameliorates the impairment of LTP induction in Aβ-infused rats. In this experiment, the slices were exposed to nicotine (50 μM) for 30 s and tetanic stimulation was applied during this period because, as mentioned above, long-term nicotine exposure reduced PS amplitude.

Application of nicotine during tetanic stimulation increased the degree of enhancement of PS amplitude induced by tetanic stimulation in vehicle control rats ($n = 7$, Fig. 4). Two-factor ANOVA analysis showed significant differences on the overall response during observation period between control slices and nicotine-treated slices, however, the amplitude at 45 min was not significant, indicating that nicotine facilitate short-term potentiation (STP, in which the enhancement persists less than 30 min) in our condition. In contrast, in Aβ-infused rats STP and/or LTP induction was not enhanced even though nicotine was contained in the perfused solution during tetanic stimulation ($F_{1,980} = 475.475$, $P < 0.01$ vs. vehicle control: $n = 4$: Fig. 4).

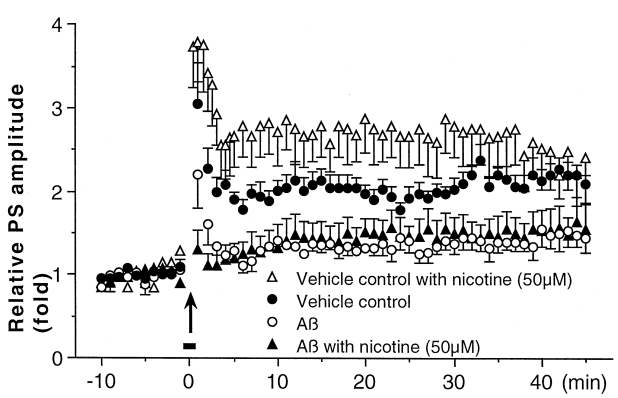

Fig. 4. Effects of nicotine on LTP induction in the hippocampal CA1 pyramidal cells of the vehicle control and Aβ-infused rats. Nicotine (50 μM) was applied for 30 s (a thick bar) and tetanic stimulation (100 Hz for 1 s), indicated as an arrow, was applied during this period. Each value represents a relative amplitude to the control level (mean ± S.E.M.). $F_{1,980} = 475.475$, $P < 0.01$ (vehicle control vs. Aβ with nicotine). The number of slices and animals were as follows: intact control $n = 3$; vehicle control $n = 7$: Aβ $n = 8$; Aβ with nicotine $n = 4$.

4. Discussion

Our previous reports indicate that Aβ is related to the learning deficits and neurodegeneration (Nabeshima and Nitta, 1994; Nitta et al., 1994, 1997), and that the Aβ-infused rat is a useful animal model of AD (Tanaka et al., 1998; Yamada et al., 1998). In this animal model, the nicotine-stimulated release of acetylcholine and dopamine in the frontal cortex/hippocampus and striatum, respectively, is also impaired (Itoh et al., 1996). In the present

study, we examined the electrophysiological properties of hippocampal neurons of intact control, vehicle control and Aβ-infused rats by using the extracellular recording technique.

First, we examined the nicotinic response in the hippocampal CA1 area, since a previous in vivo microdialysis study demonstrated decrease of nicotine-induced acetylcholine and dopamine release, suggesting the impairment of nicotinic signal transduction (Itoh et al., 1996). Although application of nicotine into the perfused solution resulted in a decrease in the electrically evoked PS amplitude in both intact and vehicle control rats, this nicotine-induced reduction was smaller in the Aβ-infused rats. This result is consistent with our previous neurochemical study that shows the decrease of nicotine-evoked release of acetylcholine and dopamine, suggesting dysfunction of nicotinic signal transduction (Itoh et al., 1996). The effect of nicotine is blocked by co-application of mecamylamine, a blocker of nicotinic acetylcholine receptor. This indicates that reduction of PS amplitude was mediated through nicotinic acetylcholine receptor, therefore, it is conceivable that Aβ impairs the function of nicotinic acetylcholine receptors, and/or the process of nicotinic signal transduction. We also found the decrease of the affinity of nicotinic acetylcholine receptor in the hippocampus of Aβ-infused rats (unpublished observation). In general, it has been thought that nicotine stimulates neurotransmitter releases via membrane depolarization induced by the opening of receptor-cation channel complexes following the binding of nicotine to nicotinic acetylcholine receptors (Brazell et al., 1990; Sargent, 1993). There are several reports showing that nicotine enhances hippocampal synaptic transmission (McGehee et al., 1995; Gray et al., 1996; Frazier et al., 1998). In contrast to our present results, Freund et al. (1988, 1990) have demonstrated that enhancement of hippocampal response induced by nicotine was due to the disruption of inhibitory GABAergic neuronal systems. In their experiments, they applied higher concentration of nicotine (800 μM) for more than 30 min. Therefore, it is conceivable that nicotinic acetylcholine receptors located on the GABAergic terminus were desensitized and then GABAergic transmission was disrupted. We applied a lower concentration of nicotine (50 μM) and exposed at a shorter time compared to the work of Freund et al. (1988, 1990), therefore, these differences may be responsible for the differences in nicotinic responses between previous and present results. The presence of presynaptic nicotinic acetylcholine receptors has been reported in the central nervous system (Clarke et al., 1984). Nicotine acting presynaptically can produce either excitation or inhibition indirectly through the release of endogenous neurotransmitters or modulators (Chesselet, 1984). Léna et al. (1993) have reported the presence of nicotinic acetylcholine receptors at a preterminal level on axons of intrinsic GABAergic neurons, and demonstrated the enhancement of the GABA releases and the increase of the frequency of GABAergic postsynaptic currents by nicotine (Limberger et al., 1986; Wonnacott et al., 1990). In our experiments, GABA antagonists blocked the nicotine-induced reduction of PS amplitude. Taken together, these findings suggest that the facilitation of the GABAergic neuronal system by stimulating nicotinic acetylcholine receptors may be responsible, at least in part, for the reduction of the PS amplitude induced by nicotine in the vehicle control rats. Since our study suggested that Aβ causes an impairment of GABAergic neuronal system and GABA might play a role in AD (Marczynski, 1998), the role of GABA in Aβ-induced brain dysfunction remains to be shown.

Next, we examined whether induction of LTP is impaired by the continuous infusion of Aβ. Although the mechanisms underlying memory storage are not well understood, LTP provides a useful cellular model for certain types of learning and memory (Bliss and Collingridge, 1993). When a tetanic stimulation was applied to the Shaffer collateral of both intact and vehicle control rats, enhanced responses of PS that were maintained for more than 45 min were observed in the hippocampal CA1 pyramidal cells. In contrast, in these areas of the Aβ-infused rats, although a transient enhancement of PS amplitude was observed immediately after the tetanic stimulation, only slight enhancement was observed. This result indicates the impairment of LTP induction in the hippocampal CA1 area of the Aβ-infused rats. Since there is general agreement that the hippocampus plays an important role in the processes of learning and memory, this deficiency may be responsible, in part, for the learning deficits in the Aβ-infused rats. Several groups have reported the effects of Aβ-related substances on LTP induction. Binsack et al. (1996) and Nalbantoglu et al. (1997) have developed transgenic mice carrying human Aβ precursor 770 and carboxy terminus of APP, respectively. They have demonstrated that not only spatial learning evaluated by water maze, but also LTP induction in the hippocampus were impaired in their transgenic animals. Cullen et al. (1997) have demonstrated that i.c.v. injection of Aβ1–40, 1–42 or C-terminal fragment greatly shortened LTP in the CA1 in vivo. Lambert et al. (1998) have also reported that application of small diffusible Aβ oligomers inhibits the induction of LTP in the medial perforant path-granule cells. Taken together with our present results, excess amount of Aβ may impair learning and memory following disruption of LTP induction. In contrast, Wu et al. (1995) and Schulz (1996) have reported that bath application of Aβ potentiated LTP in the hippocampal slices. Discrepancy between facilitatory and suppressive effects of Aβ on LTP may be explained as differences in the duration of the Aβ exposure. It has been reported that Aβ elevates intracellular Ca^{2+} concentration (Mattson et al., 1993), which plays a crucial role for LTP induction (Bliss and Collingridge, 1993). Therefore, it can be considered that acute (bath) application of Aβ potentiated LTP induction. However, as in the case of Aβ

transgenic animals, in our experiments the animals were continuously exposed to Aβ for a long period, treatment which may induce a sustained elevation of the intracellular Ca^{2+} and, therefore, may cause disruption of the neuronal functions.

In the present study, as shown in Fig. 3, we found that LTP induction was attenuated in the presence of the nicotinic blocker, hexamethonium, suggesting that nicotinic blockade is sufficient for impairment of LTP induction. Moreover, application of nicotine in the perfused solution during tetanic stimulation enhanced the degree of STP induction in the slices from vehicle control rats (Fig. 4). Enhancement of STP and/or LTP induction by nicotinic agonists has been demonstrated by Hunter et al. (1994) and Sawada et al. (1994). As yet, it has not been clearly demonstrated that nicotinic antagonists inhibit LTP induction in the hippocampal CA1 fields. However, Hunter et al. (1994) have reported that low concentration of a nicotinic agonist facilitates LTP induction in rat hippocampus, and in contrast, a high concentration inhibits LTP induction, an effect which may induce desensitization of nicotinic acetylcholine receptor. These results suggest that nicotinic receptors play a key role in LTP induction in the hippocampal CA1. Therefore, it is possible that dysfunctions of signal transduction via nicotinic receptor lead to impairment of LTP induction in Aβ-infused rats, although it is not clear how the inhibition of PS by exogenous nicotine relates to the impairments of LTP induction. The profiles of inhibition of LTP induction might be different in the slices from Aβ-infused rats and in the hexamethonium-treated slices, because the degree of LTP induction was slightly higher in hexamethonium-treated slices than in the slices from Aβ-infused rats. This suggests that the impairment of LTP induction in Aβ-infused rats may not be due to only dysfunction of nicotinic acetylcholine receptors, but also dysfunctions of other neuronal transmission. Application of nicotine during tetanic stimulation, however, failed to recover the impairment of LTP induction in Aβ-infused rats (Fig. 4). This result may be interpreted as follows: the nicotinic signal transduction is impaired in Aβ-infused rats (Fig. 1), the hippocampal CA1 cells hardly responded to nicotine. Therefore, the enhancement of STP and/or LTP induction was not observed in Aβ-infused rats in spite of the presence of nicotine. Further studies are required to elucidate the detailed mechanisms underlying neuronal dysfunction induced by continuous Aβ infusion. It is possible that Aβ causes an impairment of GABAergic neuronal system as discussed above. Further, since it is well known that NMDA receptors play an essential role in the development of LTP, we are currently investigating the function of NMDA receptors in Aβ-infused rats.

In the present study we used two different antagonists, mecamylamine and hexamethonium. Mecamylamine has been widely used to block central nicotinic acetylcholine receptors in vivo. In contrast, hexamethonium does not block central nicotinic acetylcholine receptors when administered peripherally since it cannot pass the blood–brain barrier. However, when directly administered into the brain or when incubated with brain slices in vitro, hexamethonium can block the central nicotinic acetylcholine receptors to a similar extent with mecamylamine (Kihara et al., 1997). Therefore, it is considered that both mecamylamine and hexamethonium effectively block nicotinic acetylcholine receptors in vitro. We cannot exclude the possibility, however, that two different subtypes of nicotinic acetylcholine receptors are blocked by mecamylamine and hexamethonium.

It has been reported that Aβ toxicity is potentiated by self-aggregation of Aβ 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/0.5% trifluoroacetic acid, see the experimental procedures) that would avoid such aggregation. Although acetonitrile toxicity to neuronal cells has been suggested (Waite et al., 1992), our preliminary experiments showed no differences in learning ability and ChAT activity between the acetonitrile-treated and untreated rats. This may be due to the slow infusion rate (0.5 μl h^{-1}) or to the infusion site (into the ventricle, not parenchyma). In the present results, we could not find significant differences in nicotinic response and LTP induction between the intact and vehicle control rats. Based upon these observations, it is likely that the neuronal dysfunctions found in the present and previous experiments were induced by Aβ, and not by the solvent used.

In conclusion, Aβ infusion impairs the nicotinic signal transduction mechanism at the pyramidal cell layer in the hippocampal CA1 area. In the present study, we did not investigate the contribution of other neuronal systems, however, as we previously had reported that in Aβ-infused rats not only cholinergic but also dopaminergic neuronal systems were impaired. Therefore, there is a possibility that, in the Aβ-infused rats, several neuronal systems including dopaminergic and GABAergic neuronal systems are also impaired. This dysfunction may be responsible, at least in part, for the impairment of LTP induction and leads to the impairment of learning and memory.

Acknowledgements

This study was supported in part by a grant (No. 10044260, 10897005) from the Ministry of Education, Science and Culture of Japan, an SRF Grant for Biomedical Research, and a grant from JAPS.

References

Arispe, N., Pollard, H.B., Rojas, E., 1993a. Giant multilevel cation channels formed by Alzheimer disease amyloid β-protein [AβP-(1–

40)] in bilayer membranes. Proc. Natl. Acad. Sci. U.S.A. 90, 10573–10577.

Arispe, N., Rojas, E., Pollard, H.B., 1993b. Alzheimer disease amyloid β protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminium. Proc. Natl. Acad. Sci. U.S.A. 90, 567–571.

Bierer, L.M., Haroutunian, V., Gabriel, S., Knott, P.J., Carlin, L.S., Purohit, D.P., Perl, D.P., Schmeidler, J., Kanof, P., Davis, K.L., 1995. Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. J. Neurochem. 64, 749–760.

Binsack, D.P., Lee, H.K., Leonard, C.M., Montoya-Zayala, M., Marotta, C.A., 1996. Rats transgenic for human APP770 have impaired place memory and hippocampal long-term potentiation. Neurosci. Abstr. 22, 1892.

Bliss, T.V.P., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Brazell, M.P., Mitchell, S.N., Joseph, M.H., Gray, J.A., 1990. Acute administration of nicotine increases the in vivo extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid and ascorbic acid preferentially in the nucleus accumbens of the rat: comparison with caudate–putamen. Neuropharmacology 29, 1177–1185.

Cai, X.D., Golde, T., Younkin, S., 1993. Release of excess amyloid β-protein from a mutant amyloid β-protein precursor. Science 259, 514–516.

Chartier-Harlin, M.C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J., Mullan, M., 1991. Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. Nature 353, 844–846.

Chesselet, M.F., 1984. Presynaptic regulation of neurotransmitter release in the brain: facts and hypothesis. Neuroscience 12, 347–375.

Citron, M., Oltersort, T., Haass, C., McConlogue, L., Hung, A.Y., Scubert, P., Vigo-Pelfrey, C., Lieberburg, I., Selkoe, D.J., 1992. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature 360, 672–674.

Clarke, P.B.S., Pert, C.B., Pert, A., 1984. Autoradiographic distribution of nicotine receptors in rat brain. Brain Res. 323, 390–395.

Coyle, J.T., Price, D.L., DeLong, M.R., 1983. Alzheimer's disease: cortical cholinergic innervation. Science 219, 1184–1190.

Cullen, W.K., Suh, Y.H., Rowan, M.J., 1997. Block of LTP in rat hippocampus in vivo by β-amyloid precursor protein fragment. NeuroReport 8, 3213–3217.

Davies, P., Maloney, A.J.F., 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2, 1403.

Etcheberrigaray, R., Ito, E., Oka, K., Tofel-Grehl, B., Gibson, G.E., Alkon, D.L., 1993. Potassium channel dysfunction in fibroblasts identifies patients with Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A. 90, 8209–8213.

Etcheberrigaray, R., Ito, E., Kim, C.S., Alkon, D.L., 1994. Soluble β-amyloid induction of Alzheimer's phenotype for human fibroblast K^+ channels. Science 264, 276–279.

Frazier, C.J., Rollins, Y.D., Breese, C.R., Leonard, S., Freedman, R., Dunwiddie, T.V., 1998. Acetylcholine activates an α-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. J. Neurosci. 18, 1187–1195.

Freund, R.K., Jungschaffer, D.A., Collins, A.C., Wehner, J.M., 1988. Evidence for modulation of GABAergic neurotransmission by nicotine. Brain Res. 453, 215–220.

Freund, R.K., Luntz-Leybman, V., Collins, A.C., 1990. Nicotine interferes with GABA-mediated inhibitory processes in mouse hippocampus. Brain Res. 527, 286–291.

Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., Hardy, J., 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349, 704–749.

Gray, R., Rajan, A.S., Radcliffe, K.A., Yakehiro, M., Dani, J.A., 1996. Hippocampal synaptic transmission enhanced by low concentration of nicotine. Nature 383, 713–716.

Haas, C., Schlossmacher, M.G., Hung, A.Y., Vigo-Pelfrey, C., Mellon, A., Ostaszeeski, B.L., Lieberburg, I., Koo, E.H., Schenk, D., Teplow, D.B., Selkoe, D.J., 1992. Amyloid β-peptide is produced by cultured cells during normal metabolism. Nature 359, 322–325.

Hardy, J., Allsop, D., 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Neurosci. 12, 383–388.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., Cole, G., 1996. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 274, 99–102.

Hunter, B.E., de Fiebre, C.M., Papke, R.L., Kem, W.R., Meyer, E.M., 1994. A novel nicotinic agonist facilitates induction of long-term potentiation in the rat hippocampus. Neurosci. Lett. 168, 130–134.

Itoh, A., Nitta, A., Nadai, M., Nishimura, K., Hirose, M., Hasegawa, T., Nabeshima, T., 1996. Dysfunction of cholinergic and dopaminergic neuronal systems in β-amyloid protein-infused rats. J. Neurochem. 66, 1113–1117.

Kihara, T., Shimohama, S., Sawada, H., Kimura, J., Kume, T., Kochiyama, H., Maeda, T., Akaike, A., 1997. Nicotinic receptor stimulation protects neurons against β-amyloid toxicity. Ann. Neurol. 42, 159–163.

Kosik, K.S., 1991. Alzheimer plaques and tangles: advances on both fronts. Trends Neurosci. 14, 218–219.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Walz, P., Zhang, C., Finch, C.E., Krafft, G.A., 1998. Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U.S.A. 95, 6448–6453.

Limberger, N., Späth, L., Starke, K., 1986. A search for receptors modulating the release of γ-[^{3}H]aminobutyric acid in rabbit caudate nucleus slices. J. Neurochem. 46, 1109–1117.

Léna, C., Changeux, J.P., Mulle, C., 1993. Evidence for ''preterminal'' nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. J. Neurosci. 13, 2680–2688.

Marczynski, T.J., 1998. GABAergic deafferentation hypothesis of brain aging and Alzheimer's disease revisited. Brain Res. Bull. 45, 341.

Mark, R.J., Hensley, K., Butterfield, D.A., Mattson, M.P., 1995. Amyloid β-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca^{2+} homeostasis and cell death. J. Neurosci. 15, 6239–6249.

Mattson, M.P., Barger, S.W., Cheng, B., Lieberburg, I., Smith-Swintosky, V.L., Rydel, R.E., 1993. β-amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer's disease. Trends Neurosci. 16, 409–414.

McGehee, D.S., Heath, M.J.S., Gelber, S., Devay, P., Role, L.W., 1995. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269, 1692–1696.

Murrell, J., Farlow, M., Ghetti, B., Benson, M.D., 1991. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 254, 97–99.

Nabeshima, T., 1993. Behavioral aspects of cholinergic transmission: role of basal forebrain cholinergic system in learning and memory. Prog. Brain Res. 98, 405–411.

Nabeshima, T., Nitta, A., 1994. β-amyloid protein-induced memory impairment and neuronal dysfunction animal model. Tohoku J. Med. 174, 241–249.

Nalbantoglu, J., Tirado-Santlago, G., Lahsaïni, A., Poirier, J., Goncalves, O., Verge, G., Momoli, F., Welner, S.A., Massicotte, G., Julien, J.P., Shapiro, M.L., 1997. Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. Nature 387, 500–505.

Nitta, A., Itoh, A., Hasegawa, T., Nabeshima, T., 1994. β-amyloid protein-induced Alzheimer's disease animal model. Neurosci. Lett. 170, 63–66.39.

Nitta, A., Fukuta, T., Hasegawa, T., Nabeshima, T., 1997. Continuous infusion of β-amyloid protein into the rat cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Jpn. J. Pharmacol. 73, 51–57.

Paxinos, G., Watson, C., 1986. The Rat Brain in the Stereotaxic Coordinates. Academic Press, New York.

Pike, C.J., Walencewicz, A.J., Glaba, C.G., Cotman, C.W., 1991. In vitro aging of β-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res. 563, 311–314.

Sargent, P.B., 1993. The diversity of neuronal nicotinic acetylcholine receptors. Annu. Rev. Neurosci. 16, 403–443.

Sawada, S., Yamamoto, C., Ohno-Shosaku, T., 1994. Long-term potentiation and depression in the dentate gyrus, and effects of nicotine. Neurosci. Res. 20, 323–329.

Schluz, P.E., 1996. β-peptide enhance the magnitude and probability of long-term potentiation. Neurosci. Abstr. 22, 2111.

Selkoe, D.J., 1994. Normal and abnormal biology of the β-amyloid precursor protein. Annu. Rev. Neurosci. 17, 489–517.

Shoji, M., Golde, T.E., Ghiso, J., Cheung, T.T., Estus, S., Shaffer, L.M., Cai, X.D., McKay, D.M., Tintner, R., Frangione, B., Younkin, S.G., 1992. Production of Alzheimer β protein by normal proteolytic processing. Science 258, 126–129.

Sims, N.R., Bowen, D.M., Allen, S.J., Smith, C.C., Neary, D., Thomas, D.J., Davison, A.N., 1983. Presynaptic cholinergic dysfunction in patients with dementia. J. Neurochem. 40, 503–509.

Tanaka, T., Yamada, K., Senzaki, K., Narimatsu, H., Nishimura, K., Kameyama, T., Nabeshima, T., 1998. NC-1900, an active fragment analog of arginine vasopressin, improves learning and memory deficits induced by β-amyloid protein in rats. Eur. J. Pharmacol. 352, 135–142.

Waite, J., Cole, G.M., Frautschy, S.A., Connor, D.J., Thal, L.J., 1992. Solvent effects on beta protein toxicity in vivo. Neurobiol. Aging 13, 595–599.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., DeLong, M.R., 1982. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science 215, 1237–1239.

Wilcock, G.K., Esiri, M.N., Bowen, D.M., Smith, C.C., 1982. Alzheimer's disease: correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. J. Neurol. Sci. 57, 407–417.

Wonnacott, S., Drasdo, A., Sanderson, E., Rowell, P., 1990. In: Presynaptic Nicotinic Receptors and the Modulation of Transmitter Release. Ciba Foundation Symposium. The Biology of Nicotine Dependence. Wiley, Chichester, pp. 87–105.

Wu, J., Anwyl, R., Rowan, M.J., 1995. β-amyloid-(1–40) increases long-term potentiation in rat hippocampus in vitro. Eur. J. Pharmacol. 284, R1–R3.

Yamada, K., Tanaka, T., Senzaki, K., Kameyama, T., Nabeshima, T., 1998. Propentofylline improves learning and memory deficits induced in rats by β-amyloid protein-(1–40). Eur. J. Pharmacol. 349, 15–22.

Yankner, B.A., Duffy, L.K., Kirschner, D.D., 1990. Neurotrophic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. Science 250, 279–282.