Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 583 (2008) 84–91
www.elsevier.com/locate/ejphar

Impaired muscarinic regulation of excitatory synaptic transmission in the APPswe/PS1dE9 mouse model of Alzheimer's disease

Yasuaki Goto [a], Tetsuhiro Niidome [a,*], Haruyuki Hongo [a], Akinori Akaike [b], Takeshi Kihara [a], Hachiro Sugimoto [a,*]

[a] *Department of Neuroscience for Drug Discovery, Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida-Shimoadachi-cho, Sakyo-ku, 606-8501, Japan*

[b] *Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, 606-8501, Japan*

Received 25 September 2007; received in revised form 7 January 2008; accepted 24 January 2008
Available online 5 February 2008

Abstract

Cholinergic hypothesis and amyloid cascade hypothesis are mainly proposed for Alzheimer's disease; however, the relationship between these hypotheses is poorly understood. To address the question of whether amyloid β-peptide pathology affects cholinergic neurotransmission, we examined the effect of a cholinesterase inhibitor, physostigmine, on field excitatory postsynaptic potentials (EPSPs) evoked by single-pulse stimulation in the CA1 region of the hippocampus of various APPswe/PS1dE9 transgenic mice with different degrees of amyloid β-peptide pathology. Reduced field EPSPs by physostigmine in transgenic mice at 3 months of age, when the mice had negligible amyloid β-peptide levels and no amyloid β-peptide deposits, were indistinguishable from those in age-matched wild-type mice. In contrast, reduced field EPSPs by physostigmine in transgenic mice at 5 months of age, when the mice had low amyloid β-peptide levels and subtle amyloid β-peptide deposits, were significantly lower than those in age-matched wild-type mice. Next, we characterized acetylcholine receptors, which play important roles in cholinergic neurotransmission, because physostigmine resulted in increased acetylcholine levels in the synaptic cleft. Different reductions of field EPSPs by physostigmine between transgenic and wild-type mice at 5 months of age were not affected by a nicotinic receptor antagonist, mecamylamine; however, reduced field EPSPs by physostigmine in both transgenic and wild-type mice were restored to basal levels by a muscarinic receptor antagonist, atropine. These results indicate that cholinergic modulation of glutamatergic transmission is already impaired at the onset of the formation of amyloid β-peptide deposits, and muscarinic receptor dysfunction is one of the causes of this impairment.

Keywords: Acetylcholine; Alzheimer's disease; Amyloid β-peptide; Muscarinic acetylcholine receptor; Field excitatory postsynaptic potentials

1. Introduction

Cholinergic neurotransmission in the brain is involved in learning and memory (Parent and Baxter, 2004). Clinical and post mortem studies have revealed that the cognitive impairment observed in patients with Alzheimer's disease is partly caused by the progressive loss of basal forebrain cholinergic neurons, leading to the cholinergic hypothesis of Alzheimer's disease (Bartus et al., 1982). This neuronal loss is correlated by reductions in acetylcholine levels, the number of acetylcholine receptors, and the activity of choline acetyltransferase which is an enzyme responsible for the synthesis of acetylcholine (Araujo et al., 1988). In particular, the activity of choline acetyltransferase is highly correlated with clinical dementia ratings across the neocortex of patients with Alzheimer's disease (Bierer et al., 1995). The cholinergic hypothesis is also supported by the fact that several cholinesterase inhibitors (donepezil, rivastigmine and galantamine) are currently marketed for the treatment of Alzheimer's disease (Ibach and Haen, 2004). Besides the cholinergic hypothesis, it is now generally accepted that the accumulation of amyloid β-peptide is closely related to the pathogenesis of Alzheimer's disease (the amyloid cascade hypothesis). Amyloid β-peptide, which is a 39–43-amino-acid peptide, is a product of the cleavage of amyloid

* Corresponding authors. Tel.: +81 75 753 9271; fax: +81 75 753 9269.
E-mail addresses: tniidome@pharm.kyoto-u.ac.jp (T. Niidome), hsugimot@pharm.kyoto-u.ac.jp (H. Sugimoto).

0014-2999/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.01.030

precursor protein by β- and γ-secretase (Selkoe, 2000). Previous studies have revealed that (i) point mutations of amyloid precursor protein, which are linked to familiar Alzheimer's disease, increase the relative production of amyloid β-peptide 42 (Price and Sisodia, 1998), (ii) administration of amyloid β-peptide is neurotoxic *in vitro* and *in vivo* (Kowall et al., 1991; Pike et al., 1991), and (iii) administration of anti-amyloid β-peptide antibody decreased senile plaques and cognitive impairment in an animal model of Alzheimer's disease (Schenk et al., 1999). It was demonstrated that amyloid β-peptide in nanomolar concentrations inhibits various cholinergic neurotransmitter functions (e.g., acetylcholine release and choline acetyltransferase activity) independently of its apparent neurotoxicity (Auld et al., 1998), suggesting that the impairment of cholinergic neurotransmission observed in the patients with Alzheimer's disease is partly caused by the inhibition of cholinergic markers by amyloid β-peptide. It was also demonstrated that stimulation of muscarinic acetylcholine receptors leads to non-amyloidogenic cleavage of amyloid precursor protein (Buxbaum et al., 1992; Nitsch et al., 1992), suggesting that the accelerated progression of amyloid β-peptide accumulation observed in patients with Alzheimer's disease is partly caused by the impairment of cholinergic neurotransmission. Thus, the precise relationship between amyloid β-peptide pathology and cholinergic neurotransmission is poorly understood.

Transgenic mice overexpressing familial Alzheimer's disease mutation forms of amyloid precursor protein provide a unique opportunity to characterize the time course of amyloid β-peptide pathology. Transgenic mice overexpressing amyloid precursor protein with Swedish mutation and presenilin-1 with deletion of exon 9 (APPswe/PS1dE9) at 3 months of age have negligible amyloid β-peptide levels and no amyloid β-peptide deposits (Goto et al., unpublished data). APPswe/PS1dE9 transgenic mice at 5 months of age have low amyloid β-peptide levels and subtle amyloid β-peptide deposits, whereas APPswe/PS1dE9 transgenic mice at 9 months of age have high amyloid β-peptide levels and numerous amyloid β-peptide deposits (Taniuchi et al., 2007). Previous studies have demonstrated that both choline uptake and acetylcholine receptors binding were reduced in Tg2576 transgenic mice overexpressing amyloid precursor protein with Swedish mutation (Apelt et al., 2002; Klingner et al., 2003), and both choline acetyltransferase activity and muscarinic receptor binding were reduced in APPswe/PS1dE9 transgenic mice (Machová et al., in press). However, no publication has focused on the electrophysiological properties of cholinergic neurotransmission. Therefore, in order to address the question of whether amyloid β-peptide pathology affects cholinergic neurotransmission, we first examined the effect of a cholinesterase inhibitor, physostigmine, on field excitatory postsynaptic potentials (EPSPs) evoked by single-pulse stimulation in the CA1 region of the hippocampus of various APPswe/PS1dE9 transgenic mice at 3, 5 and 9 months of age with different degrees of amyloid β-peptide pathology. Interestingly, physostigmine reduced field EPSPs in APPswe/PS1dE9 transgenic mice in an age-related manner, and the change in cholinergic modulation of glutamatergic transmission between APPswe/PS1dE9 transgenic mice and age-matched wild-type mice had already occurred at the onset of the formation of amyloid β-peptide deposits. We also examined the effects of nicotinic and muscarinic receptor antagonists on reduced field EPSPs by physostigmine in order to elucidate the cellular mechanism causing different cholinergic modulation of glutamatergic transmission between APPswe/PS1dE9 transgenic mice and age-matched wild-type mice.

2. Materials and methods

2.1. APPswe/PS1dE9 transgenic mice

APPswe/PS1dE9 transgenic mice (Jankowsky et al., 2001) were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained by crossing transgenic mice with B6C3F1 mice. The genotyping for APPswe/PS1dE9 transgenic mice was performed by the PCR method recommended by the Jackson Laboratory. All animals were housed in an air-conditioned room which was kept at a constant ambient temperature of 24±2 °C under a 12-h light/12-h dark cycle with free access to food and water. The use of experimental animals in this study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee, and the guidelines of the Japanese Pharmacological Society.

2.2. Preparation of hippocampal slices

Mice were sacrificed by decapitation after anesthesia with diethyl ether, and the whole brain was removed. The brain from transgenic or wild-type littermate mice was immediately soaked for 3 min in ice-cold artificial cerebrospinal fluid containing (in mM) 124 NaCl, 3 KCl, 26 $NaHCO_3$, 2 $CaCl_2/2H_2O$, 1 $MgSO_4/7H_2O$, 1.25 KH_2PO_4, 10 D-glucose. Appropriate portions of the brain were trimmed and placed on the ice-cold stage of a vibrating tissue slicer (VT-1000S; Leica, Nussloch, Germany). The thickness of each tissue slice was 300 μm. Hippocampal formation was incubated at 27.5 °C for 1 h in oxygenated artificial cerebrospinal fluid. A slice was then placed on the center of a 10% poly-L-lysine-coated multi-electrode dish (MED probe; Alpha MED Science, Osaka, Japan). This device has an array of 64 planar microelectrodes, each having a size of 50×50 μm, arranged in an 8×8 pattern with interpolar spacing of 150 μm.

2.3. Electrophysiological recordings

During electrophysiological recordings, the slices on the MED probe were placed in a small CO_2 incubator at 32 °C. Oxygenated and fresh artificial cerebrospinal fluid was infused at 1.0 ml/min. Evoked field potentials at all 64 sites were recorded with a multichannel recording system (MED64 system; Alpha MED Science) at a 20 kHz sampling rate and simultaneously filtered through a 100 Hz band pass filter. One of the planar microelectrodes of the 64 available was used as a stimulating cathode. To collect typical responses in field CA1, one of the electrodes in the Schaffer collateral/commissural fibers was selected as a stimulating electrode,

while another in the stratum radiatum (dendritic region) was selected as a recording electrode. In each experiment, maximal field potential was first determined by gradually increasing stimulus intensity until the saturation level was reached. Stimulus intensity was then decreased to evoke a test response that was approximately 30% of the maximal signal amplitude. The recorded field potentials were confirmed to be field EPSPs by paired-pulse facilitation. Control field EPSPs were recorded for 20 min before drug treatment. An acetylcholinesterase inhibitor, physostigmine (Sigma, St. Louis, MO), muscarinic acetylcholine receptor antagonist, atropine (Sigma), and nicotinic acetylcholine receptor antagonist, mecamylamine (Sigma), were dissolved in artificial cerebrospinal fluid. The concentrations of physostigmine, atropine and mecamylamine in this study are determined by the potency of these drugs against cholinergic neurotransmission as reported previously (Misgeld et al., 1989; Zhang and Warren, 2002).

2.4. Western blot analysis

Mice hippocampal tissues were homogenized in 10 mM HEPES buffer (pH 7.9) containing 10 mM KCl, 1 mM EDTA, 1 mM EGTA, protease inhibitor cocktail (Nacalai Tesque, Inc., Kyoto, Japan), phosphatase inhibitor cocktail (Sigma), followed by the addition of sodium dodecyl sulfate (SDS)-containing buffer with 5% mercaptoethanol at a volume ratio of 1:1 and subsequent boiling at 95 °C for 5 min. An aliquot of 10 μg protein was loaded on a 5–20% gradient SDS-polyacrylamide gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) containing 0.1% SDS for electrophoresis at a constant current of 40 mA/plate for 1 h at room temperature, and subsequently blotted onto a nitrocellulose polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA) treated previously with 100% methanol. After blocking with 5% skim milk dissolved in 20 mM Tris–HCl buffer (pH 7.5) containing 137 mM NaCl and 0.1% Tween 20, the membrane was reacted with antibodies against nicotinic receptor subunit α_4 (1:1000, Santa Cruz), nicotinic receptor subunit α_7 (1:500, Santa Cruz), muscarinic receptor subunit M_1 (1:1000, Santa Cruz), muscarinic receptor subunit M_3 (1:500, Santa Cruz) and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) (1:10,000, Ambion, Austin, TX), adequately diluted with buffer containing 5% skim milk, followed by horseradish peroxidase-conjugated anti-mouse IgG sheep antibody (1:2000; Amersham Biosciences, Buckinghamshire, UK), and horseradish peroxidase-conjugated anti-goat IgG donkey antibody (1:2000; Santa Cruz). Bands were visualized by enhanced chemiluminescence (ECL detection kit; Amersham Biosciences) on X-Omat Blue films (Kodak, Tokyo, Japan). Densitometric analysis was carried out using NIH image and normalized to the GAPDH internal control.

2.5. Statistical analysis

Data are shown as the mean ± S.E.M. Statistical comparisons were made using Student's *t*-test or Aspin–Welch's *t*-test using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Results were considered significant at $P<0.05$.

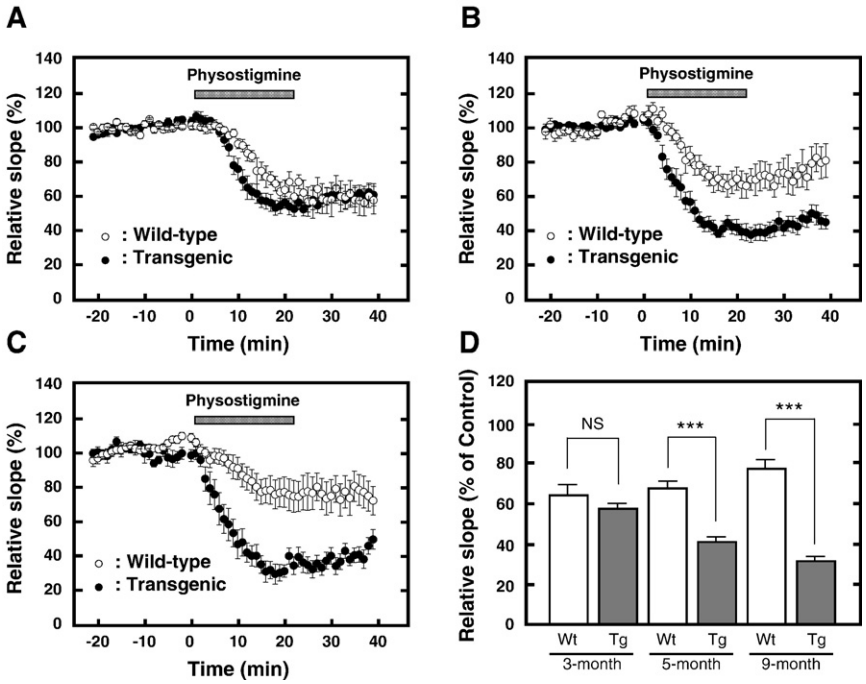

Fig. 1. Effect of physostigmine on field EPSPs in APPswe/PS1dE9 transgenic and wild-type mice. Field EPSPs evoked by stimulation of the Schaffer collateral/commissural fibers in the CA1 stratum radiatum in slices from transgenic and wild-type mice at 3 (A), 5 (B) and 9 (C) months of age were recorded. Cholinesterase inhibitor, physostigmine, at 10 μM was added to hippocampal slices for 20 min. (D) Summary of reduced field EPSPs by physostigmine. Averaged EPSP slope values from 18 to 20 min were used for statistical analysis. Control shows the averaged EPSP slope values from −2 to 0 min. Each value represents the mean ± S.E.M. ($n=7$–14 slices from 4–7 animals). ***$P<0.001$. NS: not significant. Wt: wild-type. Tg: transgenic.

3. Results

3.1. Age-related changes of cholinergic modulation of glutamatergic transmission

In order to determine whether amyloid β-peptide pathology affects cholinergic neurotransmission, we first examined the effects of a cholinesterase inhibitor, physostigmine, on field EPSPs in female transgenic and wild-type mice at 3, 5 and 9 months of age. Female mice were used as they showed faster development of amyloid β-peptide pathology than male mice (Taniuchi et al., 2007). Field EPSPs were evoked by single-pulse stimulations in Schaffer collateral/commissural fibers in the stratum radiatum of CA1. Baseline field EPSPs were recorded for 20 min before the addition of 10 μM physostigmine, which showed a similar inhibitory effect on field EPSPs among wild-type mice at 3, 5 and 9 months of age (Fig. 1). In contrast, physostigmine reduced field EPSPs to 54.4±4.7, 43.6±4.1 and 31.4±5.1% in transgenic mice at 3, 5 and 9 months of age, respectively. Thus, physostigmine significantly reduced field EPSPs in transgenic mice at 5 months of age compared to those in wild-type mice (Fig. 1D), indicating that the change in cholinergic modulation of glutamatergic transmission had already occurred at the onset of the formation of amyloid β-peptide deposits. Next, we examined the effects of physostigmine on paired-pulse facilitation in order to elucidate the primary site of action of physostigmine. Paired-pulse facilitation is a form of presynaptic short-term plasticity of excitatory synapses of the hippocampus that is related to neurotransmitter release processes (Zucker, 1989). Following two stimulations in rapid succession, the amplitude of the second field EPSPs elicited by the pair of stimuli is increased with respect to the first field EPSPs at a short interpulse interval in the CA1 region of the hippocampus. In both transgenic and wild-type mice at 5 months of age, paired-pulse facilitation was observed for stimuli applied at 50 ms intervals (Fig. 2A). Physostigmine significantly increased the paired-pulse ratio (slope of second response/slope of first response) in wild-type mice from 136.7±3.2 to 161.3±3.7%, and also significantly increased the paired-pulse ratio in transgenic mice from 145.9±3.6 to 162.7±4.8% (Fig. 2B). Thus, paired-pulse facilitation in the presence of physostigmine in transgenic mice is indistinguishable from that in wild-type mice, suggesting that at least presynaptic events contribute to reduce field EPSPs by physostigmine.

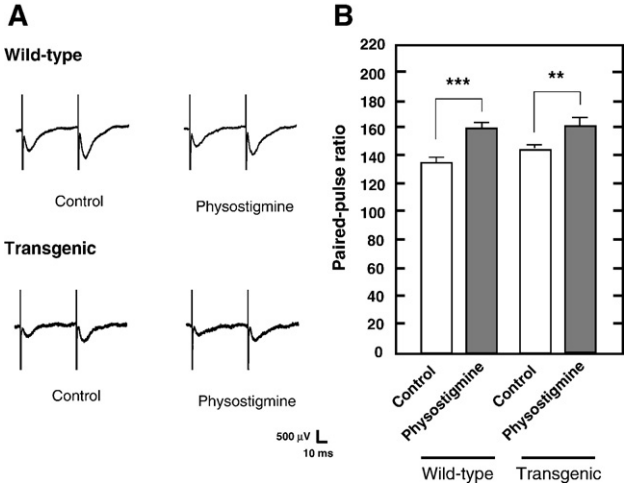

Fig. 2. Effect of physostigmine on paired-pulse facilitation in APPswe/PS1dE9 transgenic and wild-type mice. Cholinesterase inhibitor, physostigmine, at 10 μM was added to hippocampal slices for 20 min and paired-pulse stimulations were delivered to Schaffer collateral/commissural fibers with an interpulse interval of 50 ms. (A) Traces showing typical paired-pulse facilitation in wild-type mice (upper) and transgenic mice (lower) at 5 months of age. Traces were recorded in the absence (control) or presence of physostigmine. (B) The ratio of second field EPSPs/first field EPSPs of paired-pulse facilitation. Each value represents the mean±S.E.M. (n=3–4 slices from 6–8 animals). **P<0.01 and ***P<0.001.

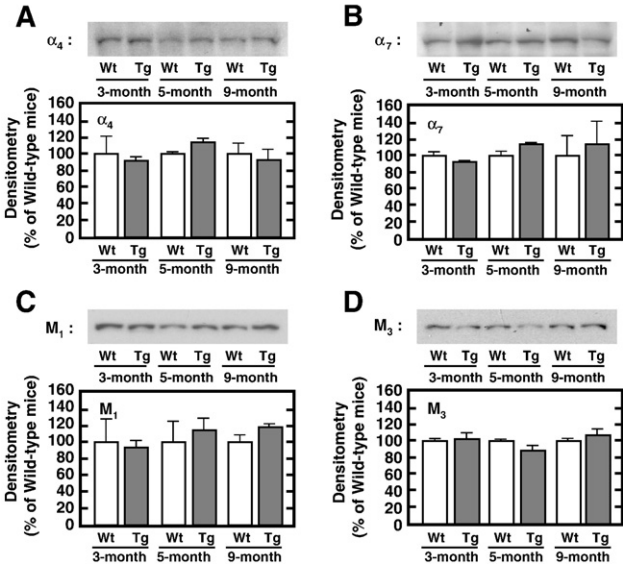

Fig. 3. Expression of nicotinic and muscarinic acetylcholine receptors in APPswe/PS1dE9 transgenic and wild-type mice. Hippocampal tissues from transgenic and wild-type mice were excised and subjected to Western blot analysis using an antibody against α_4-nicotinic receptor (A), α_7-nicotinic receptor (B), muscarinic M_1 receptor (C) or muscarinic M_3 receptor (D). GAPDH was used as an internal control (data not shown). Typical immunoblots are shown in the upper panel. The intensity of the bands was quantified and expressed as a percentage of the intensity of the bands from wild-type mice (the lower panel). Each value represents the mean±S.E.M. from 4 independent experiments. Wt: wild-type. Tg: transgenic.

3.2. Expression levels of nicotinic and muscarinic acetylcholine receptors

Next, we characterized acetylcholine receptors which play important roles in cholinergic neurotransmission, because physostigmine resulted in an increase of acetylcholine levels in the synaptic cleft. Acetylcholine receptors are classified as nicotinic and muscarinic acetylcholine receptors. Nicotinic receptors are composed of an assembly between eight α (α_2–α_9) and three β (β_2–β_4) subunits as pentameric structures, and α_4- and α_7-nicotinic receptors are expressed abundantly in the brain (Gotti et al., 1997; Weiland et al., 2000). Muscarinic receptors are composed of five distinct subtypes (M_1–M_5), and M_1 and M_3 receptors are expressed abundantly in the hippocampus of the brain (Levey, 1993). Therefore, we examined the expression

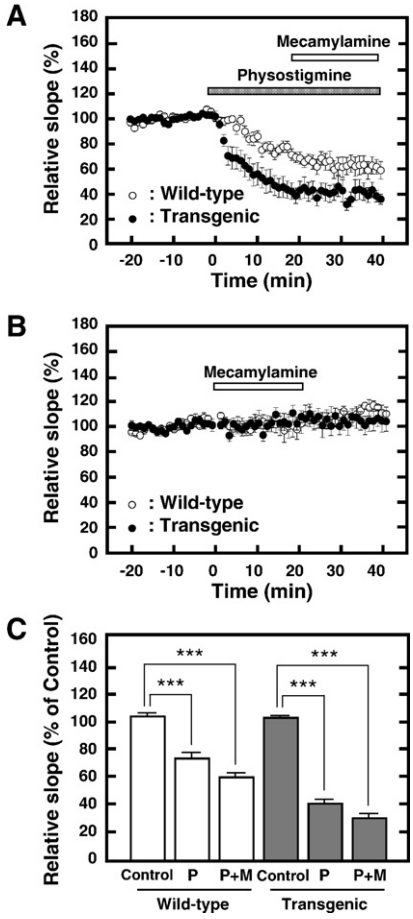

Fig. 4. Effect of nicotinic acetylcholine receptor antagonist on reduced field EPSPs by physostigmine in APPswe/PS1dE9 transgenic and wild-type mice. Stimulation and recording condition like in Fig. 1. Nicotinic acetylcholine receptor antagonist, mecamylamine, at 10 μM was added to hippocampal slices in the presence (A) or absence (B) of physostigmine. Each value represents the mean±S.E.M. (n=6–10 slices from 3–4 animals). (C) Summary of reduced field EPSPs by physostigmine. Averaged EPSP slope values from −2 to 0 min (control), from 18 to 20 min (physostigmine) and from 38 to 40 min (physostigmine plus mecamylamine) were used for statistical analysis. Each value represents the mean±S.E.M. (n=6–14 slices from 3–7 animals). ***P<0.001. P: physostigmine. P+M: physostigmine plus mecamylamine.

levels of two nicotinic receptors (α_4 and α_7) and two muscarinic receptors (M_1 and M_3) in the hippocampus of transgenic and wild-type mice at 3, 5 and 9 months of age. Unexpectedly, Western blot analyses revealed no significant change in the expression levels of α_4-nicotinic receptor, α_7-nicotinic receptor, muscarinic M_1 receptor and muscarinic M_3 receptor in transgenic mice at 3, 5 and 9 months of age, compared to age-matched wild-type mice (Fig. 3). These results suggest that the expression level of acetylcholine receptors is not a cause of the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice.

3.3. Mechanism causing the change in cholinergic modulation of glutamatergic transmission

Although expression levels of nicotinic and muscarinic acetylcholine receptor were normal in transgenic mice at 5 and 9 months of age when the mice showed different cholinergic modulation of glutamatergic transmission compared to age-matched wild-type mice, it is possible that functional impairment of acetylcholine receptor reduces field EPSPs by physostigmine. Therefore, we examined the effects of a nicotinic receptor antagonist, mecamylamine, or a muscarinic receptor antagonist, atropine, on the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice. Field EPSPs in transgenic and wild-type mice at 5 months of age were recorded from −20 min to 40 min after the addition of physostigmine. Mecamylamine or atropine was added at 20 min after the addition of physostigmine. Mecamylamine at 10 μM did not affect the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice (Fig. 4). Interestingly, atropine at 10 μM restored the reduced field EPSPs by physostigmine to the basal level in both transgenic and wild-type mice (Fig. 5A, C). Neither mecamylamine nor

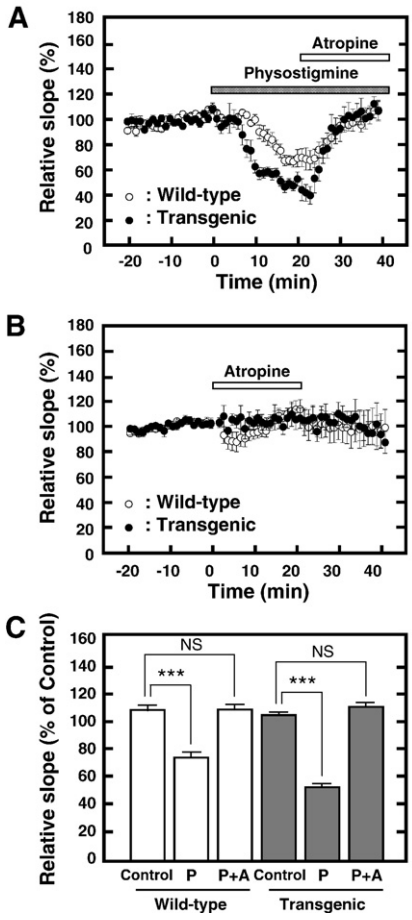

Fig. 5. Effect of muscarinic acetylcholine receptor antagonist on reduced field EPSPs by physostigmine in APPswe/PS1dE9 transgenic and wild-type mice. Stimulation and recording condition like in Fig. 1. Muscarinic acetylcholine receptor antagonist, atropine, at 10 μM was added to hippocampal slices in the presence (A) or absence (B) of physostigmine. Each value represents the mean± S.E.M. (n=6–10 slices from 3–4 animals). (C) Summary of reduced field EPSPs by physostigmine. Averaged EPSP slope values from −2 to 0 min (control), from 18 to 20 min (physostigmine) and from 38 to 40 min (physostigmine plus atropine) were used for statistical analysis. Each value represents the mean±S.E.M. (n=6–14 slices from 3–7 animals). ***P<0.001. NS: not significant. P: physostigmine. P+A: physostigmine plus atropine.

atropine at 10 μM affected field EPSPs (Figs. 4B and 5B). These results suggest that the functional impairment of muscarinic acetylcholine receptors is one of causes of the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice.

4. Discussion

In this study, we explored the relationship between two hypotheses of Alzheimer's disease: the cholinergic hypothesis and the amyloid cascade hypothesis. First, we found that reduced field EPSPs by physostigmine were indistinguishable between transgenic and wild-type mice at 3 months of age; however, they were different at 5 and 9 months of age. Second, we found that paired-pulse facilitation in the presence of physostigmine was indistinguishable between transgenic and wild-type mice at 5 months of age. Third, we found no significant change in the expression levels of α_4-nicotinic receptor, α_7-nicotinic receptor, muscarinic M_1 receptor and muscarinic M_3 receptor in transgenic mice at 3, 5 and 9 months of age, compared to age-matched wild-type mice. Finally, we found that the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice at 5 months of age was not affected by a nicotinic receptor antagonist, mecamylamine; however, reduced field EPSPs by physostigmine in both transgenic and wild-type mice were restored to basal levels by a muscarinic receptor antagonist, atropine. Considering the different degrees of amyloid β-peptide pathology of APPswe/PS1dE9 transgenic mice at 3, 5 and 9 months of age, we report here for the first time that the impairment of cholinergic modulation of glutamatergic transmission observed by electrophysiological recording techniques had already occurred at the onset of the formation of amyloid β-peptide deposits, and muscarinic receptor dysfunction is one of the causes of this impairment.

The hippocampus receives a moderately dense cholinergic projection from the medial septum and basal forebrain (Lewis and Shute, 1967; Mosko et al., 1973; Woolf et al., 1984), and has both nicotinic and muscarinic acetylcholine receptors in its target zones (Levey et al., 1995; Albuquerque et al., 1997). A non-selective cholinergic agonist, carbachol, at 5 μM significantly reduced the slope of field EPSPs evoked by single-pulse stimulations in the CA1 region of the hippocampus from both transgenic and wild-type mice (data not shown). Cholinesterase inhibitor, physostigmine, also significantly reduced the slope of field EPSPs, and thereby reduced field EPSPs are deemed to be a consequence of the enhancement of cholinergic neurotransmission. Reduced field EPSPs in transgenic mice at 3 months of age, when the mice had negligible amyloid β-peptide levels and no amyloid β-peptide deposits, were indistinguishable from those in age-matched wild-type mice; however, reduced field EPSPs in transgenic mice at 5 months of age, when the mice had low amyloid β-peptide levels and subtle amyloid β-peptide deposits, were already evident compared to those in age-matched wild-type mice. These results indicate that the impairment of cholinergic modulation of glutamatergic transmission observed in APPswe/PS1dE9 transgenic mice is associated with amyloid β-peptide pathology. It is possible that low concentrations of soluble amyloid β-peptide impair cholinergic neurotransmission as reported previously (Auld et al., 1998). In addition, it would be interesting to compare the accumulation of intraneuronal amyloid β-peptide and/or amyloid β-peptide oligomers in addition to the tissue distribution of amyloid β-peptide deposits between transgenic mice at 3 and 5 months of age.

A previous study demonstrated that reduced field EPSPs were mainly due to the activation of presynaptic muscarinic acetylcholine receptors in the presence of carbachol in micromolar concentrations (Auerbach and Segal, 1996). This is consistent with our results that paired-pulse facilitation in both transgenic and wild-type mice was enhanced by physostigmine, indicating that reduced field EPSPs are likely due to the inhibition of presynaptic glutamate release as a change in paired-pulse facilitation has been suggested to indicate a presynaptic mechanism (Zucker, 1989). Next, in order to elucidate the cellular mechanisms causing different reductions of field EPSPs between transgenic and wild-type mice, we characterized acetylcholine receptors, because physostigmine increased in acetylcholine levels in the synaptic cleft. There are two principal classes of acetylcholine receptors, nicotinic receptors and muscarinic receptors. Nicotinic receptors are ligand-gated ion channels, whereas muscarinic receptors are G protein-coupled receptors. Nicotine has been reported to modulate glutamatergic synaptic transmission in the CA3 region of the hippocampus (Gray et al., 1996; Giocomo and Hasselmo, 2005). There is a significant loss of high-affinity nicotinic receptors in the brain of patients with Alzheimer's disease (Whitehouse and Au, 1986), and the administration of nicotine patches improves impaired learning and attention in patients with Alzheimer's disease (Grigoryan et al., 1994; Newhouse et al., 1997). Muscarinic receptors have been reported to modulate glutamatergic synaptic transmission in the CA1 region of the hippocampus (Kremin et al., 2006). The administration of muscarinic receptor agonist rescued the cognitive deficits of a spatial task in a transgenic animal model of Alzheimer's diseases (Caccamo et al., 2006). A nicotinic receptor antagonist, mecamylamine, did not affect the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice (Fig. 4); however, a muscarinic receptor antagonist, atropine, rescued the reduced field EPSPs by physostigmine to basal levels in both transgenic and wild-type mice (Fig. 5), suggesting that the different reduction of field EPSPs by physostigmine between transgenic and wild-type mice is due to the impairment of presynaptic muscarinic receptors. It was reported that the inhibition of glutamatergic transmission through presynaptic muscarinic receptors plays a vital role in preventing interference from old memories during the encoding of new information in models of hippocampal circuits (Hasselmo et al., 1995; Hasselmo, 1999); however, excess inhibition of glutamate release may induce the impairment of memory and learning. There are several possible cellular mechanisms of reduced field EPSPs by muscarinic receptors. First, the activation of muscarinic receptors on glutamatergic terminals directly inhibits glutamatergic transmission by decreasing glutamate release (Hamam et al., 2007).

Second, the activation of muscarinic receptors indirectly inhibits glutamatergic transmission by increasing GABA release from interneurons (Caillard et al., 1998; Molyneaux and Hasselmo, 2002). Alternatively, the inhibition of postsynaptic muscarinic receptors which potentiate NMDA currents results in a decrease of glutamatergic transmission (Marino et al., 1998). Recently, Machova et al. reported that the coupling of muscarinic receptors with G-proteins estimated by carbachol-stimulated GTP-γ^{35}S binding was reduced in APPswe/PS1dE9 transgenic mice at 7 months of age compared to age-matched wild-type mice, indicating the impairment of muscarinic transmission (Machová et al., in press). Thus, it is possible that both the suppression of postsynaptic muscarinic receptors and enhancement of presynaptic receptors mediate the impairment of glutamatergic transmission at the onset of the formation of amyloid β-peptide deposits. Our results do not rule out other possibilities that reductions in acetylcholine release, acetylcholine levels and choline acetyltransferase activity are causes of the impairment of cholinergic neurotransmission as reported previously (Auld et al., 1998; Ikarashi et al., 2004; Hartmann et al., 2004).

In conclusion, we demonstrated here that a change in cholinergic modulation of glutamatergic transmission between APPswe/PS1dE9 transgenic mice and wild-type mice already occurs at the onset of the formation of amyloid β-peptide deposits by electrophysiological recording. Our results provide fundamental insights useful for therapeutic interventions for Alzheimer's disease.

References

Albuquerque, E.X., Pereira, E.F., Alkondon, M., Schrattenholz, A., Maelicke, A., 1997. Nicotinic acetylcholine receptors on hippocampal neurons: distribution on the neuronal surface and modulation of receptor activity. J. Recept. Signal Transduct. Res. 17, 243–266.

Apelt, J., Kumar, A., Schliebs, R., 2002. Impairment of cholinergic neurotransmission in adult and aged transgenic Tg2576 mouse brain expressing the Swedish mutation of human beta-amyloid precursor protein. Brain Res. 953, 17–30.

Araujo, D.M., Lapchak, P.A., Robitaille, Y., Gauthier, S., Quirion, R., 1988. Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. J. Neurochem. 50, 1914–1923.

Auerbach, J.M., Segal, M., 1996. Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. J. Physiol. 492, 479–493.

Auld, D.S., Kar, S., Quirion, R., 1998. Beta-amyloid peptides as direct cholinergic neuromodulators: a missing link? Trends Neurosci. 21, 43–49.

Bartus, R.T., Dean III, R.L., Beer, B., Lipa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–417.

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.

Buxbaum, J.D., Oishi, M., Chen, H.I., Pinkas-Kramarski, R., Jaffe, E.A., Gandy, S.E., Greengard, P., 1992. Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. Proc. Natl. Acad. Sci. U. S. A. 89, 10075–10078.

Caccamo, A., Oddo, S., Billings, L.M., Green, K.N., Martinez-Coria, H., Fisher, A., LaFerla, F.M., 2006. M1 receptors play a central role in modulating AD-like pathology in transgenic mice. Neuron 49, 671–682.

Caillard, O., McLean, H.A., Ben-Ari, Y., Gaiarsa, J.L., 1998. Ontogenesis of presynaptic GABAB receptor-mediated inhibition in the CA3 region of the rat hippocampus. J. Neurophysiol. 79, 1341–1348.

Giocomo, L.M., Hasselmo, M.E., 2005. Nicotinic modulation of glutamatergic synaptic transmission in region CA3 of the hippocampus. Eur. J. Neurosci. 22, 1349–1356.

Gotti, C., Fornasari, D., Clementi, F., 1997. Human neuronal nicotinic receptors. Prog. Neurobiol. 53, 199–237.

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

Grigoryan, G.A., Peters, S., Gray, J.A., Hodges, H., 1994. Interactions between the effects of propranolol and nicotine on radial maze performance of rats with lesions of the forebrain cholinergic projection system. Behav. Pharmacol. 5, 265–280.

Hamam, B.N., Sinai, M., Poirier, G., Chapman, C.A., 2007. Cholinergic suppression of excitatory synaptic responses in layer II of the medial entorhinal cortex. Hippocampus 17, 103–113.

Hartmann, J., Erb, C., Ebert, U., Baumann, K.H., Popp, A., Konig, G., Klein, J., 2004. Central cholinergic functions in human amyloid precursor protein knock-in/presenilin-1 transgenic mice. Neuroscience 125, 1009–1017.

Hasselmo, M.E., 1999. Neuromodulation and the hippocampus: memory function and dysfunction in a network simulation. Prog. Brain Res. 121, 3–18.

Hasselmo, M.E., Schnell, E., Barki, E., 1995. Dynamics of learning and recall at excitatory recurrent synapses and cholinergic modulation in rat hippocampal region CA3. J. Neurosci. 15, 5249–5262.

Ibach, B., Haen, E., 2004. Acetylcholinesterase inhibition in Alzheimer's disease. Curr. Pharm. Des. 10, 231–251.

Ikarashi, Y., Harigaya, Y., Tomidokoro, Y., Kanai, M., Ikeda, M., Matsubara, E., Kawarabayashi, T., Kuribara, H., Younkin, S.G., Maruyama, Y., Shoji, M., 2004. Decreased level of brain acetylcholine and memory disturbance in APPsw mice. Neurobiol. Aging 25, 483–490.

Jankowsky, J.L., Slunt, H.H., Ratovitski, T., Jenkins, N.A., Copeland, N.G., Borchelt, D.R., 2001. Co-expression of multiple transgenes in mouse CNS: a comparison of strategies. Biomol. Eng. 17, 157–165.

Klingner, M., Apelt, J., Kumar, A., Sorger, D., Sabri, O., Steinbach, J., Scheunemann, M., Schliebs, R., 2003. Alterations in cholinergic and non-cholinergic neurotransmitter receptor densities in transgenic Tg2576 mouse brain with beta-amyloid plaque pathology. Int. J. Dev. Neurosci. 21, 357–369.

Kowall, N.W., Beal, M.F., Busciglio, J., Duffy, L.K., Yankner, B.A., 1991. An in vivo model for the neurodegenerative effects of beta amyloid and protection by substance. Proc. Natl. Acad. Sci. U. S. A. 88, 7247–7251.

Kremin, T., Gerber, D., Giocomo, L.M., Huang, S.Y., Tonegawa, S., Hasselmo, M.E., 2006. Muscarinic suppression in stratum radiatum of CA1 shows dependence on presynaptic M1 receptors and is not depend on effects at GABA(B) receptors. Neurobiol. Learn. Mem. 85, 153–163.

Levey, A.I., 1993. Immunological localization of M_1–M_5 muscarinic acetylcholine receptors in peripheral tissues and brain. Life Sci. 52, 441–448.

Levey, A.I., Edmunds, S.M., Koliatsos, V., Wiley, R.G., Heilman, C.J., 1995. Expression of M_1–M_4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. J. Neurosci. 15, 4077–4092.

Lewis, P.R., Shute, C.C., 1967. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. Brain 90, 521–540.

Machová, E., Jakubík, J., Michal, P., Oksman, M., Iivonen, H., Tanila, H., Doležal, V., in press. Impairment of muscarinic transmission in transgenic APPswe/PS1dE9 mice. Neurobiol. Aging. doi:10.1016/j.neurobiolaging.2006.10.029.

Marino, M.J., Rouse, S.T., Levey, A.I., Potter, L.T., Conn, P.J., 1998. Activation of the genetically defined M_1 muscarinic receptor potentiates *N*-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells. Proc. Natl. Acad. Sci. U. S. A. 95, 11465–11470.

Misgeld, U., Müller, W., Polder, H.R., 1989. Potentiation and suppression by eserine of muscarinic synaptic transmission in the guinea-pig hippocampal slice. J. Physiol. 409, 191–206.

Molyneaux, B.J., Hasselmo, M.E., 2002. GABA(B) presynaptic inhibition has an in vivo time constant sufficiently rapid to allow modulation at theta frequency. J. Neurophysiol. 87, 1196–1205.

Mosko, S., Lynch, G., Cotman, C.W., 1973. The distribution of septal projections to the hippocampus of the rat. J. Comp. Neurol. 152, 163–174.

Newhouse, P.A., Potter, A., Levin, E.D., 1997. Nicotinic system involvement in Alzheimer's and Parkinson's diseases. Implications for therapeutics. Drugs Aging 11, 206–228.

Nitsch, R.M., Slack, B.E., Wurtman, R.J., Growdon, J.H., 1992. Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science 258, 304–307.

Parent, M.B., Baxter, M.G., 2004. Septohippocampal acetylcholine: involved in but not necessary for learning and memory? Learn. Mem. 11, 9–20.

Pike, C.J., Walencewicz, A.J., Glabe, C.G., Cotman, C.W., 1991. Aggregation-related toxicity of synthetic beta-amyloid protein in hippocampal cultures. Eur. J. Pharmacol. 207, 367–368.

Price, D.L., Sisodia, S.S., 1998. Mutant genes in familial Alzheimer's disease and transgenic models. Annu. Rev. Neurosci. 21, 479–505.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vandevert, C., Walker, S., Wogulis, M., Yednock, T., Games, D., Seubert, P., 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400, 173–177.

Selkoe, D.J., 2000. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. Ann. N.Y. Acad. Sci. 924, 17–25.

Taniuchi, N., Niidome, T., Goto, Y., Akaike, A., Kihara, T., Sugimoto, H., 2007. Decreased proliferation of hippocampal progenitor cells in APPswe/PS1dE9 transgenic mice. NeuroReport 18, 1801–1805.

Weiland, S., Bertrand, D., Leonard, S., 2000. Neuronal nicotinic acetylcholine receptors: from the gene to the disease. Behav. Brain Res. 113, 43–56.

Whitehouse, P.J., Au, K.S., 1986. Cholinergic receptors in aging and Alzheimer's disease. Prog. Neuropsychopharmacol. Biol. Psychiatry 10, 665–676.

Woolf, N.J., Eckenstein, F., Butcher, L.L., 1984. Cholinergic systems in the rat brain: I. projections to the limbic telencephalon. Brain Res. Bull. 13, 751–784.

Zhang, L., Warren, R.A., 2002. Muscarinic and nicotinic presynaptic modulation of EPSCs in the nucleus accumbens during postnatal development. J. Neurophysiol. 88, 3315–3330.

Zucker, R.S., 1989. Short-term synaptic plasticity. Annu. Rev. Neurosci. 12, 13–31.