Effect of Subchronic Treatment of Memantine, Galantamine, and Nicotine in the Brain of Tg2576 (APPswe) Transgenic Mice

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ABSTRACT

An increasing number of studies suggest that the present clinical therapy used in Alzheimer’s disease (AD), in addition to having a symptomatic effect, also may interact with the ongoing neuropathological processes in the brain. The aim of this study was to investigate the effect of the cholinesterase inhibitor galantamine and the N-methyl-D-aspartate (NMDA) antagonist memantine in comparison to nicotine on the neuropathology of Tg2576 transgenic mice (APPswe). Nontransgenic and APPswe mice at 10 months of age were treated subcutaneously with saline, memantine, galantamine, or nicotine for 10 days. Nicotine reduced the guanidinium-soluble amyloid-β peptide (Aβ) levels by 46 to 66%, whereas the intracellular Aβ levels remained unchanged. Treatment with nicotine also resulted in less glial fibrillary acidic protein immunoreactive astrocytes around the plaques, increased levels of synaptophysin, and increased number of α7 nicotinic acetylcholine receptors (nAChRs) in the cortex of APPswe transgenic mice. Galantamine treatment caused an increase in the cortical levels of synaptophysin in the APPswe mice. Memantine treatment reduced the total cortical levels of membrane-bound amyloid precursor protein (45–55%) in both transgenic and nontransgenic mice, which eventually may decrease the level of Aβ. In conclusion, galantamine, memantine, and nicotine have different interactions with Aβ processes, α7 nAChRs, and NMDA receptors in APPswe mice. These different effects might have therapeutic relevance, and this knowledge might be applicable to the development of new effective therapeutic strategies for AD.

Alzheimer’s disease (AD) is a progressive neurodegenerative disease with declarative memory impairments and increasingly severe dementia. The pathological hallmarks in post-mortem AD brains are neuritic plaques and neurofibrillary tangles with gliosis as well as neuron and synapse loss, primarily in the hippocampus and cortical brain regions (Cummings and Cole, 2002). Cortical and hippocampal synapse density is reduced early in the disease process, and the loss of these synapses correlates strongly with memory impairments (Terry et al., 1991). There is a substantial loss of cholinergic innervation in the cerebral cortex and hippocampus (Coyle et al., 1983), as well as a severe loss of the neurotransmitter acetylcholine and the neuronal nicotinic acetylcholine receptors (nAChRs), mainly in the cortical regions and the hippocampus (Martin-Ruiz et al., 1999; Guan et al., 2000). The nAChRs are recognized as playing an important role in different processes including neurite outgrowth, synaptic transmission, cognitive function, control and synthesis of neurotrophic factors, and neuroprotection (Donnelly-Roberts, 1998; Belluardo et al., 2000; Hernandez and Terry, 2005). The changes in the cholinergic transmission are accompanied by a number of alterations in other neurotransmitter systems (Nordberg, 1992).

Different treatment strategies have been explored in AD (Nordberg, 2003), and the available therapy today is focused on enhancement of the cholinergic neurotransmission or modulation of the glutamatergic neurotransmission. A therapeutic benefit in cognition, with functional and behavioral improvements, has been observed by treatment with the AChEI galantamine (Tariot et al., 2000), and memantine, a noncompetitive voltage-dependent reversible blocker of N-methyl-D-aspartate (NMDA) receptors (Reisberg et al., 2003). Both compounds exhibit neuroprotective effects in vitro (Par-
sons et al., 1999; Arias et al., 2004) as well as in vivo neuronal death models (Parsons et al., 1999; Capsoni et al., 2002).

One of the major targets in treatment strategies today is to lower the amyloid-β peptide (Aβ) load in the AD brain (Cummings, 2004). By affecting the production, aggregation, or clearance of Aβ, a modifying effect on disease progression is expected. The Tg2576 (APPsw) transgenic mice carrying the human amyloid precursor protein (APP<sub>G70R71</sub>) mutation show early phenotypic changes, which in part reflect AD pathology (Hsiao et al., 1996). By using this APP animal model, it is possible to investigate the effect of compounds that may inhibit Aβ accumulation or influence other neuro-pathological markers in the brain. Recent studies in these mice have revealed that both short- and long-term treatment with nicotine reduce the levels of insoluble Aβ1–40 and Aβ1–42 in the brain of APPsw mice (Nordberg et al., 2002; Hellström-Lindahl et al., 2004).

Preclinical studies suggest that AChEIs may attenuate neuronal damage and death from cytoxic insults, thereby affecting the AD pathogenesis (Francis et al., 2005). The aim of this study was to investigate the effect of treatment with galantamine, memantine, as well as nicotine, on the different neuropathological changes in APPsw transgenic mice.

**Materials and Methods**

**Materials**

[^3H]Dizocilpine hydrogen maleate (MK-801) (specific activity 17.1 Ci/mmol) and [125I]-α-bungarotoxin (specific activity 148 Ci/mmol) were purchased from DuPont/NEN (Boston, MA). Autoradiographic microstandards and [3H]-Hyperfilm were obtained from Amersham Life Science (Uppsala, Sweden). D-19 developer and Fixer solution were purchased from Kodak (Stockholm, Sweden). Memantine hydrochloride and (−)-nicotine were obtained from Sigma Aldrich (Stockholm, Sweden), whereas galantamine hydrobromide was a gift from Janssen-Cilag (Stockholm, Sweden). Antibodies for Western blot were purchased from Chemicon International (Temecula, CA) (22C11), DakoCytomation (Stockholm, Sweden) (Saprophytisphynx), and Santa Cruz Biotechnology (Santa Cruz, CA) (donkey anti-mouse and goat anti-rabbit). All other chemicals were of analytical grade.

**Mice**

The Principles of Laboratory Animal Care (National Institutes of Health publication 86-23, revised 1985) were followed. All animal experimental protocols were approved by the local Ethics Committee and carried out in accordance with the guidelines of the Swedish National Board for Laboratory Animals (Dnr S81/01).

Tg2576 mice overexpressing APP<sub>695</sub> and containing a KM670/671 mutation driven by a hamster prion protein gene promoter in C57B6 × SJL F1 hybrid mice were back-crossed to C57B6 mice (Hsiao et al., 1996), and C57B6 males (Bomice and Mollegaard Breeding Laboratories, Ejby, Denmark) were used to breed a colony under the same conditions. The mice had access to food and water ad libitum and were maintained on a 12-h light/dark cycle. Ten-month old APP<sub>695</sub> mice and nontransgenic controls were randomized into four age groups, 2, 3, 6, and 10 months old, and carried out in accordance with the guidelines of the Swedish National Board for Laboratory Animals (Dnr S81/01). The mice were weighed at day 0 and day 10. At day 0, the mean weight of the APPsw transgenic mice was significantly less (31%) compared with the nontransgenic controls. There were no differences in weight between the treatment groups in either the APPsw transgenic or the nontransgenic control mice. No significant difference in weight from baseline (day 0) could be observed in any of the APPsw or nontransgenic control treatment group at day 10. Blood was taken intracardially from each mouse 1 h after the last injection (plasma was obtained by centrifugation), and then the mice were sacrificed by decapitation. The brain tissue was quickly removed and cut in half sagittally. One half was immediately frozen on dry ice, whereas the other half was dissected at 4°C with the aim to obtain the cortex. The tissue was then frozen and maintained at −80°C until use.

**Galantamine and Memantine Concentration in Plasma**

Galantamine and memantine concentration in plasma was determined by high-pressure liquid chromatography following detection by mass spectrometry. Data are presented as the mean nanograms per milliliter plasma concentration in APPsw and nontransgenic controls.

**Analysis of Aβ1–40 and Aβ1–42 Concentrations in the Cortex**

Cortical tissue was homogenized in 7 volumes of 20 mM Tris-HCl, pH 8.5, containing protease inhibitor cocktail (Complete; Roche Diagnostics Scandinavia AB, Bromma, Sweden). The homogenate was then divided (1/4 to ELISA and 3/4 to Western blot). The homogenate used for ELISA was then centrifuged at 100,000 × g for 1 h at 4°C, and the supernatant was diluted 1:4 with PBS including 0.5% bovine serum albumin, 0.05% Tween 20, and protease inhibitors (standard buffer) and used for analysis of soluble Aβ. The pellet was extracted in 10 volumes of 5 M guanidine HCl in 20 mM Tris-HCl, pH 8.0, for 1.5 h in room temperature and then diluted 1:10 with standard buffer and centrifuged at 13,100 g for 20 min at 4°C. The supernatant was used for measurements of insoluble Aβ1–40 and Aβ1–42.

The level of Aβ1–40 and Aβ1–42 peptides was analyzed by using the Signal Select. Human β-amyloid 1–40 and 1–42 colorimetric sandwich ELISA kits (BioSource International Inc., Camarillo, CA) were used as previously described (Hellström-Lindahl et al., 2004).

**Immunohistochemistry**

Double immunofluorescence labeling of Aβ and glial fibrillary acidic protein (GFAP) was performed by pretreating the sections with concentrated formic acid for 5 min, followed by incubation of the sections in 5% normal goat serum in Tris-buffered saline. Then, the sections were incubated with a cocktail of primary antibodies (anti-6E10/anti-GFAP) overnight at 4°C, followed by a secondary antibody cocktail consisting of Cy2-conjugated goat anti-mouse and Cy3-conjugated goat anti-rabbit antibodies (each 20 μg/ml; Chemicon). For control staining, the primary serum was omitted and resulted in no detectable labeling.
The APPswe transgenic mice start to develop plaques around 9 months of age (Hsiao et al., 1996). Therefore, only a few plaques per slide were found in the cortex of these mice. These plaques were examined to determine the effect of the different treatment on aggregated Aβ. Immunoreactive material in the brain sections was observed using an Axioshot microscope (Carl Zeiss AG, Göttingen, Germany) equipped with a digital camera (Hamamatsu Photonics, Hamamatsu City, Japan).

### Tissue Preparation for Western Blot

Cortices from five mice of each treatment group were used. Homogenates were prepared with ice-cold 20 mM Tris-HCl, pH 8.5, containing Roche Diagnostics protease inhibitor cocktail and then centrifuged at 60,000g for 20 min. The resulting pellets were resuspended in the same buffer with the addition of 2% Triton X-100. The suspension was mixed for 2 h at 4°C and then centrifuged at 100,000g for 1 h. The supernatant was used for Western blot analysis of total membrane-bound APP and synaptophysin. The DC protein assay kit (Bio-Rad, Stockholm, Sweden), using bovine serum albumin as a standard, was used to measure the protein content in the fractions.

### Western Blot

Total APP expression was quantified by immunoblotting with the monoclonal antibody 22C11, which recognizes both human and mouse APP. Synaptophysin was quantified by immunoblotting as previously described (Unger et al., 2005a). Briefly, 4 to 20% gradient minigels were run at 100-mV constant voltages at room temperature for 60 to 70 min. The protein bands were blotted on a polyvinylidene difluoride membrane (RPN 303 F; Amersham Life Science) at 4°C for 90 min. The membranes were blocked overnight at 4°C. The blots were incubated with the appropriate secondary antibody, donkey anti-mouse and goat anti-rabbit (1:1000), for 1 h at room temperature. The strips were then incubated with the APP (1:500) or the synaptophysin antibody (1:2000) for 1 h at room temperature. Then, each membrane was incubated with the appropriate secondary antibody, donkey anti-mouse and goat anti-rabbit (1:1000), for 1 h at room temperature. The blots were incubated with ECL Plus reagents (Amersham Life Science), exposed to film, and developed according to standard procedures.

### Image Analysis of Western Blot

The films were scanned using a Sharp JX-325 scanner (Pharmacia Biotech, Uppsala, Sweden). The optical density values of the bands were calculated as a product of contour optical density and the area of the contour using Image Master 1D software (version 1.10; Pharmacia Biotech, Uppsala, Sweden). All samples from one treatment group were loaded on the same gel with a pooled sample of all groups as a reference. All samples were then standardized to this pooled sample, allowing for comparison between groups (Unger et al., 2005b).

### Receptor Autoradiography Assays

**[3H]MK-801 Autoradiography.** NMDA receptor autoradiography was measured using 10 nM [3H]MK-801 (DuPont NEN, Boston, MA) and 10-μm frozen tissue sections as previously described (Unger et al., 2005b). Nonspecific binding was assessed in the presence of 10 μM (+)-MK-801 (Sigma Aldrich).

**125I-α-Bungarotoxin Autoradiography.** Nicotinic receptor autoradiography was measured using 1.79 nM [125I]-α-bungarotoxin (DuPont NEN) and 10-μm frozen tissue sections as previously described (Unger et al., 2005b). Nonspecific binding was assessed in the presence of 1 μM α-bungarotoxin (Sigma Aldrich).

### Image Analysis of Autoradiography and Phosphorimaging

The autoradiography films ([3H]MK-801 and [125I]-α-bungarotoxin) were analyzed as previously described (Unger et al., 2005b).

### Statistical Analysis

Statistical analyses of the autoradiographic and Western blot data were carried out using the nonparametric Kruskal-Wallis test followed by Mann-Whitney to calculate the significance of APPswe and nontransgenic controls. The same tests were used to calculate the significance between treatment with galantamine, nicotine, and memantine on APPswe and nontransgenic mice, respectively.

### Results

#### Effect of Treatment on Cortical Membrane-Bound APP Levels

To investigate whether treatment with galantamine, memantine, or nicotine could influence the total membrane-bound levels of APP in the cortex, we used the antibody 22C11, which detects both mouse and human total APP. A 2-fold (p < 0.01) significant increase in APP levels was observed in the cortex of APPswe mice compared with the nontransgenic controls (Fig. 1). A significant decrease in total APP levels by 45% (p < 0.05) and 55% (p < 0.05) was observed following memantine treatment in APPswe transgenic mice and nontransgenic controls, respectively. Nicotine treatment significantly lowered the total APP levels by 35% (p < 0.05) in the cortex of nontransgenic mice compared with saline-treated animals, whereas no change was observed in the APPswe transgenic mice.

#### Analysis of Aβ1–40 and Aβ1–42 Concentrations in the Cortex

The specified ELISA measured the levels of human Aβ1–40 and Aβ1–42 in the cortex of APPswe transgenic mice. No signal for human Aβ could be detected in the cortex of the nontransgenic mice. No significant change in Tris-soluble (soluble) Aβ1–40 was observed following treatment with memantine, galantamine, or nicotine (Fig. 2). Tris-soluble Aβ1–42 was below detection levels.

A significant reduction in guanidinium-soluble (insoluble) Aβ1–40 by 46% (p < 0.05) and Aβ1–42 levels by 66% (p < 0.05) was observed following treatment with nicotine compared with saline-treated animals (Fig. 2). Treatment with memantine or galantamine did not significantly reduce the...
insoluble Aβ levels compared with saline-treated animals (Fig. 2).

Immunohistochemistry of Aβ and GFAP

The regional Aβ plaque formation and activated cortical astrocytes were assayed by immunofluorescence labeling with mouse antibody 6E10 directed toward Aβ1–17 and rabbit antibody directed toward GFAP (Fig. 3). Extracellular Aβ plaques, associated with GFAP immunoreactive astrocytes, as well as intracellular Aβ were detected in the cortex of APPswe transgenic mice treated with saline (Fig. 3, A and B). After treatment with nicotine, no extracellular amyloid plaques were found in the cortex and no GFAP immunoreactive astrocytes associated with the plaques were detected (Fig. 3). Remaining intracellular Aβ was observed. No effect on the Aβ plaque formation or the GFAP immunoreactive astrocytes was observed following treatment with galantamine or memantine compared with saline-treated APPswe transgenic mice.

Synaptophysin Levels in the Cortex

To reveal whether enhanced expression of the human APP induces abnormalities in the synapses, the levels of the synaptic marker synaptophysin were determined in the cortex of APPswe mice and nontransgenic controls. A significant decrease in the levels of synaptophysin by 77% (p < 0.05) was observed in the cortex of APPswe mice compared with nontransgenic controls (Fig. 4). Galantamine and nicotine treatment caused a significant increase (2-fold) (p < 0.05) of the synaptophysin levels in the APPswe mice compared with saline-treated animals (Fig. 4). No effect following memantine treatment was observed on the levels of synaptophysin in the cortex of APPswe transgenic animals or nontransgenic controls.

Autoradiography

NMDA Receptor Binding Sites. The regional distribution of NMDA receptors was determined by in vitro autora-
diography using the radioligand [3H]MK-801 in the brain of APPswe and nontransgenic controls. A significant increase in the number of [3H]MK-801 binding sites was observed in the CA2 (21%) ($p < 0.05$) and CA3 (28%) ($p < 0.05$) of the hippocampus, the temporal/parietal cortex (19%) ($p < 0.05$), and the caudate nucleus (33%) ($p < 0.01$) of the APPswe mice compared with the nontransgenic controls (Table 1). An increase in [3H]MK-801 binding sites was observed in the caudate nucleus in the nontransgenic mice following treatment with galantamine (27%) ($p < 0.05$) and nicotine (35%) ($p < 0.05$) respectively, compared with saline-treated animals (Table 1). Treatment with galantamine caused a significant decrease in the number of [3H]MK-801 binding sites in almost all brain regions investigated except for the CA3, frontal cortex, and thalamus, in the APPswe mice compared with saline-treated mice. No effect of memantine treatment was observed on the [3H]MK-801 binding sites in either the APPswe mice or the nontransgenic controls.

### Table 1

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Saline (n = 9)</th>
<th>Memantine (n = 6)</th>
<th>Nicotine (n = 9)</th>
<th>Galantamine (n = 5)</th>
<th>[3H]MK-801 (fmol/mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>26.93</td>
<td>18.30</td>
<td>17.31</td>
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<td>24.49</td>
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<tr>
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<td>31.38</td>
<td>32.00</td>
</tr>
<tr>
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<td>28.00</td>
<td>28.93</td>
<td>31.38</td>
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<tr>
<td>Thalamus</td>
<td>51.03</td>
<td>51.03</td>
<td>51.03</td>
<td>51.03</td>
<td>51.03</td>
</tr>
<tr>
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<td>7.31</td>
<td>7.31</td>
<td>7.31</td>
<td>7.31</td>
</tr>
<tr>
<td>Retrosplenial cortex</td>
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<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
</tr>
<tr>
<td>Temporal/parietal cortex</td>
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<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
</tr>
<tr>
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</tr>
<tr>
<td>苍白球</td>
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<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
</tr>
<tr>
<td>Caudate</td>
<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
<td>31.90</td>
</tr>
<tr>
<td>Thalamus</td>
<td>51.03</td>
<td>51.03</td>
<td>51.03</td>
<td>51.03</td>
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</tr>
</tbody>
</table>

### Discussion

Accumulation of $\beta\beta$ in the brain is believed to play a key role in the pathological features of AD. In this study, we confirmed our observation that treatment with nicotine dramatically reduces the levels of insoluble $\beta\beta-40$ and $\beta\beta-42$ in APPswe transgenic mice (Nordberg et al., 2002; Hellström-Lindahl et al., 2004). In addition, we observed that short-term nicotine treatment, besides eliminating the plaques in the cortex of APPswe transgenic mice, also drastically reduced the number of GFAP immunoreactive astrocytes surrounding the plaques. To determine whether the clearance of $\beta\beta$ might be due to the $\beta$-sheet breaking activity of nicotine or whether nicotine can activate astrocytes and thereby promote increased clearance of $\beta\beta$ remains to be studied. Interestingly, the levels of intracellular $\beta\beta$ were not reduced, and it is therefore tempting to suggest that the action of nicotine (or one of its metabolites) might mainly be via degradation of insoluble $\beta\beta$. It was recently demonstrated that $\Lambda(-)-$nicotine not only inhibits the aggregation of $\beta\beta-40$ and $\beta\beta-42$ but can also disaggregate fibrils preformed from both of these peptides (Ono et al., 2002). Furthermore, it has also been shown that both enantiomers of nicotine ($\Lambda(\Lambda)-$ and $\Lambda(-)-$nicotine) can affect the early stages of $\beta\beta$ aggregation, delaying oligomerization and fibril formation and thereby maintaining a population of less toxic $\beta\beta$ species (Moore et al.,
2004). This suggests that this effect may not be due to a highly specific binding interaction between nicotine and Aβ, as previously thought, but could be due instead to a weaker, relatively nonspecific, binding, or it could be due to the antioxidant or metal-chelating properties of nicotine. Treatment with nicotine also significantly lowered the levels of cortical total APP compared with saline-treated animals in the non-transgenic controls. This effect was not observed in the APPswe transgenic mice, although nicotine significantly lowered the Aβ levels in these mice. Because nicotine does not change the activities of cortical α-, β-, or γ-secretase in APPswe transgenic mice or nontransgenic controls (Hellström-Lindahl et al., 2004), nicotine may act by degrading the insoluble Aβ deposits, rather than affecting the accumulation of the peptide.

Although memantine or galantamine treatment did not alter the levels of Aβ in the brain of APPswe transgenic mice, this does not exclude that the drugs may interact with Aβ processes. Thus, memantine significantly decreased cortical total APP levels in both APPswe transgenic mice and nontransgenic mice. Because APP is expressed by glutamatergic neurons (Ouimet et al., 1994), blockade of the NMDA receptors may influence the production of total APP. The levels of Aβ may eventually decrease because of a lower production of APP following long-term treatment with memantine.

The Aβ deposit is hypothesized to disrupt neural and synaptic function leading to neuronal degeneration (Cuello, 2005). Analysis of the synaptic vesicle membrane protein synaptophysin in the present study revealed a 5-fold decrease in the cortical levels in APPswe transgenic mice compared with the nontransgenic controls, which is in agreement with earlier observations in young APPswe transgenic mice (Unger et al., 2005a). The finding that treatment with galantamine and nicotine both caused a 2-fold increase in synaptophysin levels in the APPswe transgenic mice may suggest a neuroprotective effect by influencing the possible interaction between Aβ and the α7 nAChRs.

Several lines of evidence suggest that the Aβ toxicity might be related to elevated levels of glutamate and/or overactivity of the NMDA receptors. The cellular damage of AD brains is especially prominent in areas that display glutamatergic synaptic plasticity (Arendt et al., 1998). The observation that Aβ reduces long-term potentiation and facilitates long-term depression suggests a role for Aβ in regulating trafficking of glutamate receptors. In the present study, we observed a significant increase in the number of NMDA receptor binding sites in the CA2, CA3, temporal/parietal cortex, and caudate nucleus of the APPswe transgenic mice. This might reflect compensatory changes in response to the high Aβ levels in these mice. A signaling pathway where Aβ1–42 binds to α7 nAChRs may impair glutamatergic transmission, compromise synaptic function, and reduce long-term potentiation, thereby promoting endocytosis of NMDA receptors in cortical neurons as was recently reported (Snyder et al., 2005). Neuronal cell cultures from APPswe transgenic mice showed a reduction in surface-expressed NMDA receptors, whereas no change was observed in total receptor number (Snyder et al., 2005). The increase in NMDA receptors observed in the present study might therefore be the result of a compensatory response to a persistent activation of the α7 nAChRs by Aβ, which consequently affects the NMDA receptors. This mechanism needs to be further investigated. Treatment with galantamine caused a significant decrease in the number of NMDA receptor binding sites in the APPswe mice compared with saline-treated mice. Galantamine may potentiate the activity of NMDA receptors via protein kinase C (Moriguichi et al., 2004) and interact with the α7 nAChRs (Kihara et al., 2004), but the exact mechanism behind this needs to be further evaluated.

We found an increase in α7 nAChRs in the cortex of APPswe transgenic mice compared with nontransgenic controls, which is in agreement with earlier observations, and this suggests an interaction between the α7 nAChR and Aβ (Wang et al., 2000a,b; Dineley et al., 2001; Bednar et al., 2002). The α7 nAChRs are characterized by a rapid desensitization following exposure to nicotinic agonists (Alkondon et al., 1994). Similarly, it is well known that treatment with nicotinic agonists, such as nicotine itself, up-regulates the nAChRs, mainly the α4 nAChRs, whereas the α7 nAChRs are more resistant to up-regulation (Benwell et al., 1988; Breese et al., 1997; Nordberg et al., 2002; Nguyen et al., 2003; Hellström-Lindahl et al., 2004). In the present study, we observed an increase in the α7 nAChRs in the CA3 of the hippocampus and in the temporal/parietal cortex of nicotine-treated APPswe transgenic animals compared with saline-treated mice, suggesting that Aβ might potentiate the nicotine-induced desensitization of the α7 nAChRs and thereby cause an up-regulation of the α7 nAChRs. The two drugs currently used in AD therapy, the AChEI galantamine and the NMDA receptor antagonist memantine, Table 2

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Saline</th>
<th>Memantine</th>
<th>Galantamine</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-tg (n = 9)</td>
<td>APPswe (n = 5)</td>
<td>Non-tg (n = 10)</td>
<td>APPswe (n = 5)</td>
</tr>
<tr>
<td>Non-tg (n = 7)</td>
<td>APPswe (n = 5)</td>
<td>Non-tg (n = 6)</td>
<td>APPswe (n = 5)</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>6.16 ± 0.58</td>
<td>8.37 ± 0.79*</td>
<td>7.49 ± 0.62</td>
<td>8.88 ± 1.22</td>
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<tr>
<td>Frontal cortex</td>
<td>5.00 ± 0.30</td>
<td>6.34 ± 0.87</td>
<td>5.83 ± 0.58</td>
<td>6.51 ± 0.68</td>
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<tr>
<td>Retropg. cortex</td>
<td>3.14 ± 0.33</td>
<td>4.59 ± 0.79</td>
<td>4.53 ± 0.74</td>
<td>5.09 ± 0.95</td>
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<tr>
<td>Temp/parial cortex</td>
<td>6.85 ± 0.64</td>
<td>9.90 ± 0.55</td>
<td>8.28 ± 0.98</td>
<td>10.06 ± 1.42</td>
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<td>Hippocampus</td>
<td>10.82 ± 0.35</td>
<td>12.77 ± 0.93</td>
<td>11.78 ± 0.92</td>
<td>14.12 ± 1.18</td>
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<tr>
<td>CA1</td>
<td>11.85 ± 1.02</td>
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<td>CA2</td>
<td>8.38 ± 0.45</td>
<td>10.15 ± 0.89</td>
<td>9.38 ± 20.97</td>
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<td>CA3</td>
<td>14.40 ± 0.36</td>
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<td>18.56 ± 1.95</td>
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<tr>
<td>Caudate nucleus</td>
<td>2.70 ± 0.20</td>
<td>2.92 ± 0.31</td>
<td>2.47 ± 0.26</td>
<td>3.19 ± 0.31</td>
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<tr>
<td>Thalamus</td>
<td>4.59 ± 0.37</td>
<td>4.56 ± 0.26</td>
<td>4.24 ± 0.51</td>
<td>4.81 ± 0.34</td>
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<td></td>
<td>4.59 ± 0.37</td>
<td>4.56 ± 0.26</td>
<td>4.24 ± 0.51</td>
<td>4.81 ± 0.34</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. nontransgenic (Non-tg) mice in the same treatment group, and # P < 0.05 compared with saline-treated non-tg or APPswe, respectively (Kruskall-Wallis followed by Mann-Whitney post hoc test).
had in the present study different effects in the APPswe transgenic mice. Galantamine may affect the nAChRs and the NMDA receptors, thereby mediating plasticity changes in the brain. This might partly explain the neuroprotective effect seen following treatment with galantamine (Arias et al., 2004). The observation that treatment with memantine for 10 days decreased the levels of total membrane-bound APP suggests that prolonged treatment may decrease the levels of Aβ. Neither galantamine nor memantine, however, had comparable effects with nicotine in reducing insoluble Aβ levels in the APPswe transgenic mice. By reducing plaque and activated astrocytes, nicotine seems to reduce the extracellular deposits, whereas the intracellular Aβ is unchanged. Further studies on the interaction of intracellular and extracellular Aβ at synapses may lead to a better understanding of the interactive effect of nicotine on AD pathology and may promote new therapeutic strategies for the disease. One suggestion might be long-term treatment with memantine in combination with a nicotine-like drug, which might reduce both the intracellular and extracellular Aβ in the brain.

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References

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