Butyrylcholinesterase Is Associated With β-Amyloid Plaques in the Transgenic APPSWE/PSEN1dE9 Mouse Model of Alzheimer Disease

Sultan Darvesh, PhD, MD, Meghan K. Cash, BSc, George Andrew Reid, BSc, Earl Martin, MSc, Arnold Mitnitski, PhD, and Changiz Geula, PhD

Abstract
Histochemical analysis of Alzheimer disease (AD) brain tissues indicates that butyrylcholinesterase (BuChE) is present in β-amyloid (Aβ) plaques. The role of BuChE in AD pathology is unknown, but an animal model developing similar BuChE-associated Aβ plaques could provide insights. The APPSWE/PSEN1dE9 transgenic mouse (ADTg), which develops Aβ plaques, was examined to determine if BuChE associates with these plaques, as in AD. We found that in mature ADTg mice, BuChE activity associated with Aβ plaques. The Aβ-, thioflavin-S– and BuChE-positive plaques mainly accumulated in the olfactory structures, cerebral cortex, hippocampal formation, amygdala, and cerebellum. No plaques were stained for acetylcholinesterase activity. The distribution and abundance of plaque staining in ADTg closely resembled many aspects of plaque staining in AD. Butyrylcholinesterase staining consistently showed fewer plaques than were detected with Aβ immunostaining but a greater number of plaques than were visualized with thioflavin-S. Double-labeling experiments demonstrated that all BuChE-positive plaques were Aβ positive, whereas only some BuChE-positive plaques were thioflavin-S positive. These observations suggest that BuChE is associated with a subpopulation of Aβ plaques and may play a role in AD plaque maturation. A further study of this animal model could clarify the role of BuChE in AD pathology.

Key Words: β-amyloid, Alzheimer disease, Amygdala, Cerebellum, Cerebral cortex, Cholinesterases, Hippocampus, Olfactory structures, Thioflavin-S.

INTRODUCTION
Alzheimer disease (AD) is a progressive neurodegenerative disorder that causes cognitive and behavioral impair-

From the Departments of Medicine (SD, AM), and Anatomy and Neurobiology (SD, MKC, GAR), Dalhousie University; Department of Chemistry, Mount Saint Vincent University (SD, EM), Halifax, Nova Scotia, Canada; and Cognitive Neurology and Alzheimer’s Disease Center, Northwestern University, Feinberg School of Medicine, Chicago, Illinois (CG). Send correspondence and reprint requests to: Sultan Darvesh, PhD, MD, Room 1308, Camp Hill Veterans’ Memorial, 5955 Veterans’ Memorial Lane, Halifax, Nova Scotia, Canada B3H 2E1; E-mail: sultan.darvesh@dal.ca

This research was supported by the Canadian Institutes of Health Research (MO1-82798), Canadian Institutes of Health Research Vascular Health and Dementia Initiative (DOV-78344), Capital Health Research Fund, Nova Scotia Health Research Foundation, Faculty of Medicine of Dalhousie University, Multiple Sclerosis Society of Canada, and National Institutes of Neurological Disorders and Stroke (NS057429).
Swedish mutation (APP*SWE) and the human presenilin-1 gene with the exon 9 deletion variant (PSEN1dE9) (30, 31). In humans, APP*SWE (32) and PSEN1dE9 (33) are known to cause early-onset familial AD. With both mutations, this ADTg mouse develops Aβ plaques early in life, starting at 4 months, which increase in abundance with age (34–36). Therefore, this ADTg mouse was used to determine if, as in AD, BuChE activity is associated with the Aβ plaques in this mouse model of AD. We also conducted a comprehensive analysis of the regional distribution and abundance of plaques in various brain regions. In addition, because cholinesterase activity may play a role in plaque maturation from nonfibrillar Aβ plaques to those that are fibrillar-β-pleated and thioflavin-S positive, we assessed the correlation between the distribution and abundance of Aβ-, BuChE-, and thioflavin-S–positive plaques. Double-labeling experiments were also performed to confirm the association of cholinesterases with Aβ and thioflavin-S plaques.

**MATERIALS AND METHODS**

**Wild-Type and Transgenic AD Mice**

Three male C57BL/6J wild-type (WT) mice (aged 16–17 months) and 4 male B6.Cg-Tg(APP'SWE/PSEN1dE9) 85Dbo/j transgenic (ADTg) mice (aged 15–16 months) purchased from Jackson Laboratory (Bar Harbor, ME), were used in this study. Although the WT mice lack the C3H/HeJ part of the strain background found in the ADTg mice, the WT mice were used to demonstrate the absence of Aβ- and cholinesterase-associated plaques; with respect to our experimental procedures, therefore, this strain was considered appropriate. Animals were cared for according to the guidelines set by the Canadian Council on Animal Care. Formal approval to conduct the experiments was obtained from the Dalhousie University Committee on Laboratory Animals.

Mice were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (200 mg/kg) and perfused with approximately 25 mL of 0.9% saline solution containing 0.1% sodium nitrite followed by 50 mL of 0.1 mol/L phosphate buffer ([PB] pH 7.4) containing 4% paraformaldehyde. Brains were removed and postfixed in PB containing 4% paraformaldehyde for 1–2 hours. Tissue was cryoprotected and stored in PB containing 30% sucrose and 0.1% sodium azide. Brains were cut in 30-μm serial sections in a coronal plane on a Leica SM2000R microtome (Leica Microsystems Inc., Nussloch, Germany) with Physitemp freezing stage and BFS-30TC controller (Physitemp Instruments, Inc., Clifton, NJ). Adjacent sections were stained for Aβ using an immunohistochemical technique for fibrillar Aβ with thioflavin-S histochemistry or for AChE or BuChE by histochemical techniques using choline thioester substrates. Sections were also double-labeled with BuChE staining and thioflavin-S, BuChE staining and Aβ immunostaining, or Aβ immunostaining and thioflavin-S.

**Aβ Immunohistochemistry**

Briefly, sections were rinsed for 5 minutes in 0.05 mol/L PB (pH 7.4), followed by distilled water (dH₂O), and then gently shaken in 90% formic acid for 2 minutes to immunohistochemical staining (37). Sections were rinsed 5 times in dH₂O for 1 minute each and 2 times in PB for 15 minutes. Sections were placed in 0.3% H₂O₂ in PB for 30 minutes to quench endogenous peroxidase activity and rinsed again for 30 minutes in PB. Sections were then incubated in PB containing 0.1% Triton X-100, normal goat serum (1:100), and a polyclonal rabbit anti-amyloid antibody (1:400; 71-5800, Invitrogen, Camarillo, CA), specific for the 4- to 5-kDa amyloid peptide derived by cleavage from the amyloid precursor protein (38) for approximately 16 hours at room temperature. After rinsing, sections were incubated in PB with 0.1% Triton X-100, biotinylated goat anti-rabbit secondary antibody (1:500), and normal goat serum (1:1000) for 1 hour. After another rinse, sections were placed in PB with 0.1% Triton X-100 and Vectastain Elite ABC kit (1:182; PK-6100, Vector Laboratories, Burlingame, CA), according to the manufacturer’s instructions for 1 hour. Sections were rinsed and developed in a solution of PB containing 1.39 mmol/L 3,3′-diaminobenzidine tetrahydrochloride (DAB). After 5 minutes, 50 μL of 0.3% H₂O₂ in dH₂O was added per milliliter of DAB solution, and the sections were incubated for 10 minutes. The reaction was stopped by rinsing the sections in 0.01 mol/L acetate buffer (pH 3.3). In control experiments, no staining was observed when the primary antibody was omitted.

**Thioflavin-S Histofluorescence Staining**

Sections were mounted onto glass slides, air-dried overnight, rehydrated in dH₂O, dehydrated in a series of ethanol washes, cleared in xylene, and rinsed in 50% ethanol. Sections were then incubated for 15 minutes in a solution of 0.05% thioflavin-S in 50% ethanol, rinsed in 80% ethanol and dH₂O, and coverslipped with an aqueous mounting medium. Thioflavin-S histofluorescence staining method was the same for human tissue except that before the final rinse in dH₂O, sections were placed in a solution of 0.3% Sudan black in 70% ethanol for 1 minute to quench autofluorescence, which is prevalent in human brain tissue.

**Cholinesterase Histochemistry**

Cholinesterase histochemical staining was performed using a modified Karnovsky-Roots method (39, 40). All reagents were purchased from Sigma-Aldrich (St. Louis, MO). The substrate used for visualization of BuChE activity was butyrylthiocholine, where AChE activity was inhibited by BW 284 C 51 (1,5-bis [4-allyl dimethylammonium phenyl] pentane-3-one dibromide) at a final concentration of 0.01 mmol/L. Briefly, tissue sections were rinsed in 0.1 mol/L of maleate buffer (pH 7.4) for 30 minutes; then they were then incubated for 1 hour 45 minutes in 0.1 mol/L of maleate buffer (pH 6.8) containing 0.5 mmol/L of sodium citrate, 0.47 mmol/L of cupric sulfate, 0.05 mmol/L of potassium ferricyanide, 0.8 mmol/L of butyrylthiocholine iodide, and 0.01 mmol/L of BW 284 C 51. All sections were then rinsed with gentle agitation for 30 minutes in dH₂O and placed in 0.1% cobalt chloride in water for 10 minutes. After further rinsing in dH₂O, sections were placed in PB containing 1.39 mmol/L of DAB. After 5 minutes in the DAB solution, 50 μL of 0.15% H₂O₂ in dH₂O was added per milliliter of DAB solution, and the reaction was carried out for approximately 3 minutes. Sections
were then washed in 0.01 mol/L acetate buffer (pH 3.3), mounted on slides, coverslipped, and examined with bright-field microscopy.

The procedure for the visualization of AChE activity was similar to that for BuChE, except that the substrate used was acetylthiocholine iodide at a concentration of 0.4 mmol/L and BuChE activity was inhibited with 0.06 mmol/L ethopropazine. The sections were incubated with the substrate for approximately 5 minutes. The procedure for visualization of BuChE and AChE in human tissue was the same as that for mouse except that sections were incubated in the Karnovsky-Roots solution for 2 hours and 1.5 hours, respectively.

Control experiments were performed to determine the specificity of the staining methods for BuChE and AChE. For these, the histochemical procedures were carried out in the absence of the choline thioester substrates to determine if the staining reagents used reacted with endogenous tissue components. No staining was detected in these control experiments. When butyrylthiocholine was used as the substrate with the BuChE inhibitor ethopropazine, very light staining characteristic of AChE activity was observed. When acetylthiocholine was used as the substrate with the AChE inhibitor, BW 284 C 51, the staining characteristic of BuChE was observed. When acetylthiocholine was used as the substrate in the presence of the AChE inhibitor BW 284 C 51 plus the BuChE inhibitor ethopropazine, the very light staining characteristic of AChE was observed. When butyrylthiocholine was used as the substrate in the presence of the AChE inhibitor BW 284 C 51 plus the BuChE inhibitor ethopropazine, no staining was observed. When butyrylthiocholine was used as the substrate with the AChE inhibitor BW 284 C 51, specific BuChE staining was observed. When acetylthiocholine was used as the

**FIGURE 1.** (A - D) Photomicrographs showing plaque staining for β-amyloid (A), thioflavin-S (B), and butyrylcholinesterase (C) in the entorhinal cortex of a 15-month-old APPSWE/PSEN1dE9 mouse. (D) There is no staining of plaques for acetylcholinesterase but the neuropil is stained. Insets in each panel are high-power photomicrographs demonstrating plaque morphologies in the cortex (Ai and ii; Bi and ii; Ci and ii) and white matter (Ai-iii, Bi-iii, Ci-iii). Scale bars = 100 μm and 50 μm (insets).
substrate with the BuChE inhibitor ethopropazine, specific AChE staining was observed.

**Double-Labeling Studies**

Double-labeling procedures were performed to determine the relationship between BuChE-positive and Aβ- and thioflavin-S–positive plaques. For Aβ and thioflavin-S double staining, sections were first stained with thioflavin-S, photographed, and then stained with Aβ immunohistochemistry for fluorescence microscopy. Briefly, sections already stained for thioflavin-S (as described above) were rinsed for 5 minutes in 0.05 mol/L of PB, pH 7.4, followed by dH2O. Sections were gently shaken in 90% formic acid for 2 minutes, followed by 5 rinses in dH2O for 1 minute each and 2 rinses in PB for 15 minutes each. Sections were then incubated in PB containing 0.1% Triton X-100, normal donkey serum (1:100), and rabbit anti-Aβ antibody (1:200; 71-5800, Invitrogen, Camarillo, CA) for approximately 16 hours at room temperature. After rinsing, sections were incubated in PB with 0.1% Triton X-100, donkey anti-rabbit Alexa Fluor 555 secondary antibody (1:250; A-31572, Invitrogen), and normal donkey serum (1:1000) for 1 hour. Sections were rinsed in PB, mounted on slides, and coverslipped.

For BuChE and Aβ double labeling, sections were first stained for BuChE and then stained for Aβ using immunohistochemistry for fluorescence microscopy, as described above. Sections were mounted, coverslipped, and photographed. For BuChE and thioflavin-S double labeling, sections were first stained for BuChE, followed by thioflavin-S, using the same procedures as for individual staining.

**FIGURE 2.** (A–D) Photomicrographs showing plaque staining for β-Amyloid (Aβ) (A), thioflavin-S (B), butyrylcholinesterase (BuChE) (C), and acetylcholinesterase (AChE) (D) in the entorhinal cortex from Alzheimer disease brain tissue. Insets in each panel are high-power photomicrographs demonstrating plaque morphologies. Note the similar plaque staining with Aβ, BuChE, AChE, and thioflavin-S. The tissue examined was from an 87-year-old woman with progressive dementing illness (cause of death, congestive heart failure; postmortem interval, 6.5 hours) and fulfilled neuropathological criteria for Alzheimer disease. Brain tissue provided by the Maritime Brain Tissue Bank (Halifax, Nova Scotia, Canada). Scale bars = 100 μm and 50 μm (inset).
Data Analysis

The stained mouse brain sections were analyzed and photographed using a Zeiss Axioplan 2 motorized microscope with a Zeiss AxioCam HRc digital camera using AxioVision 4.6 software (Carl Zeiss Canada Ltd., Toronto, Ontario, Canada). The photographs were assembled using Adobe Photoshop. The images were color balanced, contrast enhanced, and the brightness was adjusted to match the background from different images. In double-labeling procedures, 2 photographs were taken in the same area of interest for each stain. These photographs were digitally superimposed using Adobe Photoshop.

The distribution of Aβ-, BuChE-, and thioflavin-S-positive plaques in various areas of the ADTg mouse brain was determined; on average, 7 sections were analyzed for plaque load for each area studied. In addition, the relative abundance of each plaque marker was measured in each section, and correlations of abundance among the 3 markers were calculated. Plaque loads were quantified using National Institutes of Health ImageJ software and recorded as a percentage of the total area, as described elsewhere (41). Gray-scale images of adjacent sections stained for BuChE, Aβ, and thioflavin-S were taken throughout the brain. An intensity threshold level was set such that stained plaques, but not background, was selected. The areas of interest were parceled according to the mouse atlases by Paxinos and Franklin (42) and supplemented with the Allen Mouse Brain Atlas (43), outlined with the polygon selection tool and measured for the percent area covered by plaque staining. Areas containing BuChE staining associated with neuropil, white matter, or neuronal staining were excluded because the focus of the present work was to determine the comparative distribution of Aβ, BuChE, and thioflavin-S plaques. The percentage of Aβ, BuChE, or thioflavin-S plaque pathology was then averaged for each area and compared.

The associations between Aβ, BuChE, and thioflavin-S plaque loads were analyzed using Pearson correlation coefficients in each animal after averaging across the animals and using medians in each structure. The level of significance was set at 0.05.

RESULTS

Plaque Staining Characteristics

No plaque staining for Aβ, BuChE, or thioflavin-S was observed in the brain tissues of WT control mice. In contrast, in mice of comparable ages, numerous compact and diffuse plaques were detected using Aβ immunohistochemistry (Fig. 1A) and with thioflavin-S histofluorescence (Fig. 1B), as noted elsewhere (34, 36, 44–49). We extended these observations by demonstrating that plaques in the brains of ADTg mice are also associated with BuChE activity (Fig. 1C).

No plaques were detected in the brain tissue of this ADTg mouse with AChE histochemistry (Fig. 1D), as noted earlier (50). AChE-positive neuropil with altered staining characteristics was noted in the cerebral cortex in close proximity to suspected plaques (Fig. 1Di).

The presence of plaques in ADTg mice stained for BuChE, Aβ, and thioflavin-S reflects that seen in human AD brain tissue (Fig. 2A–C), and as reported elsewhere (23, 27).

On the other hand, the absence of AChE plaque staining in ADTg mice (Fig. 1D) differs from what is observed in AD brain tissue (Fig. 2D).

Distribution of Aβ-, BuChE-, and Thioflavin-S-Positive Plaques

The distribution of plaques labeled for Aβ, thioflavin-S, and BuChE was mapped in adjacent brain sections of the ADTg mice (Fig. 3). In general, all 3 markers detected plaques in the ADTg mouse brain. Aβ-Amyloid immunohistochemistry detected the largest number of plaques, with fewer plaques detected as BuChE-positive, and the least number of plaques detected with thioflavin-S staining. An analysis of the distribution of plaques stained with the

![Figure 3](http://jnen.oxfordjournals.org/)

*FIGURE 3. (A–G) β-Amyloid immunohistochemistry (left), thioflavin-S histofluorescence (middle), and butyrylcholinesterase histochemistry (right) in a 15-month-old APPswe/PSEN1dE9 mouse brain at representative rostral to caudal levels. Scale bar = 1 mm. 7N, facial nucleus; AN, anterior nucleus, amygdala; AC, anterior commissure; ADT, anterodorsal thalamic nucleus; AO, accessory olfactory bulb; Au, auditory cortex; AV, anteroventral thalamic nucleus; BMA, basomedial nucleus, amygdala; BST, bed nucleus stria terminalis; CbG, granular layer, cerebellum; CBM, molecular layer, cerebellum; CC, corpus callosum; CG, cingulate cortex; CG/RS, cingulate/retrosplenial cortex; CI, claustrum; CO, cochlear nucleus; CoA, cortical nucleus, amygdala; cp, cerebral peduncle; CuP, caudate putamen; DB, diagonal band nucleus; EC, external capsule; En, endopiriform nucleus; Ent, entorhinal cortex; EPI, external plexiform layer, olfactory bulb; EPIA, external plexiform layer, accessory olfactory bulb; f, fornix; fl, fimbria, hippocampus; GL, glomular cell layer, olfactory bulb; GP, globus pallidus; GRa, granule cell layer, accessory olfactory bulb; GRb, granular cell layer, dentate gyrus; HY, hypothalamus; i, insular cortex; IC, internal capsule; ICP, inferior cerebellar peduncle (restiform body); IPf, internal plexiform layer, olfactory bulb; IPN, interpeduncular nucleus; L, prelimbic/limbic cortex; LA, lateral nucleus, amygdala; LMo, lacunosum molecular, hippocampus; LO, lateral olfactory tract; LOT, nucleus of the lateral olfactory tract; LS, lateral septal nucleus; LV, lateral ventricle; M, motor cortex; MeA, medial nucleus, amygdala; MG, medial geniculate nucleus; Mi, mitral/tufted cell layer, olfactory bulb; MiA, mitral cell layer, accessory olfactory bulb; MfL, medial longitudinal fasciculus; MoL, molecular layer, olfactory bulb; MRN, medullary reticular nucleus; O, orbital cortex; ON, olfactory nerve layer; Opt, optic tract; OR, stratum oriens; PAG, periaqueductal gray matter; Pir, piriform cortex; PoDG, polymorphic layer of dentate gyrus; Pr, prepositional nucleus; PtA, parietal association cortex; Py, pyramidal layer, hippocampus; Py, pyramidal tract; Rad, stratum radiatum; RF, reticular formation; RN, red nucleus; RS, retrosplenial cortex; Rt, reticular thalamic nucleus; S, somatosensory cortex; SC, superior colliculus; SI, substantia innominata; sm, stria medullaris, thalamus; SN, substantia nigra; Sp5, spinal trigeminal tract; Sp5O, spinal trigeminal nucleus, oral part; st, stria terminalis; Subl, subiculum; T, thalamus; TeA, temporal association cortex; TT, taenia tecta; Tu, olfactory tubercle; v, visual cortex; V3, third ventricle; V4, fourth ventricle; Ve, vestibular nuclei; VP, ventral pallidum.*
Figure 3.
Figure 3. Continued.
3 markers is summarized in the Table. Detailed descriptions of the major areas of plaque location are given below.

### Olfactory Bulb and Primary Olfactory Cortices

The olfactory bulb and primary olfactory cortices had plaques stained for Aβ, thioflavin-S, and BuChE. In the main olfactory bulb (MOB), large numbers of plaques were located in the granular cell layer (Fig. 3A). The remaining layers of the MOB and the accessory olfactory bulb did not exhibit plaque staining.

Within the primary olfactory cortices, the endopiriform nucleus, anterior olfactory nucleus, taenia tecta, and the piriform cortex (Fig. 3B) had numerous plaques stained, whereas the olfactory tubercle had sparse plaque staining (Fig. 3C). The nucleus of the lateral olfactory tract had no staining (Fig. 3D) for any of the markers.

### Cerebral Cortex

Throughout the cerebral cortex, numerous plaques were positive for all 3 markers (Fig. 3B). This included the orbital, frontal, prefrontal/limbic, insular, motor, somatosensory, cingulate, cingulate/retrosplenial, retrosplenial, parietal association, auditory, temporal association, and visual cortices (Table). At this stage of development in this ADTg mouse, no cortical field was spared of pathology, which is in keeping with what is observed in the human AD cerebral cortex.

### Hippocampal Formation

The hippocampal formation also had numerous Aβ-, thioflavin-S-, and BuChE-positive plaques in the ADTg mice. Most were within the subiculum, entorhinal cortex, molecular and lacunosum moleculare layers, and polymorphic layer of the dentate gyrus (Fig. 3E, F). The stratum oriens, stratum radiatum, and pyramidal layer of the hippocampus proper and the granular layer of the dentate gyrus had sparse plaques (Fig. 3E, F).

### Amygdala

In the ADTg amygdala, Aβ, BuChE, and thioflavin-S plaques were located in the lateral, cortical, and basomedial...
nuclei (Fig. 3D, E), but the anterior, central, and medial nuclei (Fig. 3D, E) and the associated bed nucleus stria terminalis (Fig. 2C) did not have any stained plaques.

### Cerebellum

The cortex of the cerebellum had numerous plaques in the molecular layer but few in the granular layer (Table; Fig. 3G). None of the major nuclei of the cerebellum, namely the medial, interposed, or lateral, contained plaques.

### Subcortical Structures

There were very few plaques in the thalamus (Fig. 3D–F). In addition, sparse plaques were noted in the lateral septal nuclei (Fig. 3C), whereas no plaques were stained in the medial septal/diagonal band nuclei (Fig. 3C) or hypothalamus (Fig. 3C–F). There were scattered plaques, particularly in the ventral parts of the basal ganglia, including the caudate putamen (Fig. 3C–E); no plaques were observed in the accumbens, claustrum, globus pallidus, or ventral pallidum (Fig. 3C, D).

Within the brainstem, the inferior colliculus was the only structure containing plaques. A number of fiber tracts also stained positively for plaques. These included the lateral olfactory tract, anterior commissure, corpus callosum, and the external capsule (Fig. 3A–F).

In summary, plaques were found predominantly in certain olfactory structures, cerebral cortex, hippocampal formation, amygdala, and cerebellum of the ADTg mouse brain. There were only scattered plaques in the subcortical structures, including the brainstem. Although each plaque-containing structure had all 3 markers, there were consistent distinct differences in the relative amounts of individual markers.

### Comparison of Abundance of Aβ-, Thioflavin-S–, and BuChE-Positive Plaques

The percentages of the total area covered by each of Aβ-, thioflavin-S–, and BuChE-positive plaques were determined for each region of the brain analyzed (Table). There was a strong correlation in each region with respect to the area covered by Aβ-, thioflavin-S–, and BuChE-positive plaques. The Pearson’s correlation for Aβ and thioflavin-S was 0.89 (p < 0.01; Fig. 4A); that for Aβ and BuChE was 0.93 (p < 0.01; Fig. 4B); and that for BuChE and thioflavin-S was 0.97 (p < 0.001; Fig. 4C). Thus, when a structure had a high Aβ plaque load, there was also a correspondingly high level of thioflavin-S– and BuChE-positive plaques, with the highest correlation being between BuChE and thioflavin-S–positive plaques. Conversely, in areas with low levels of Aβ plaques, there was a correspondingly low level of thioflavin-S– and BuChE-stained plaques. Although the overall distribution of Aβ–, thioflavin-S–, and BuChE-positive plaques was analogous within each plaque-containing region (Table; Fig. 3), the percentage of the total area stained was consistently highest for Aβ, lower for BuChE, and lowest for thioflavin-S (Fig. 5).

### Double-Labeling of Plaques

Based on thioflavin-S histofluorescence, followed by Aβ immunofluorescence, all thioflavin-S–positive plaques appeared to be Aβ positive, but not all Aβ-positive plaques were stained for thioflavin-S when the 2 photomicrographs of the same section were merged (Fig. 6A–C). This is consistent with the observation that the area covered by Aβ was always higher than that covered by thioflavin-S (Table; Fig. 5).

---

**FIGURE 4.** (A–C) Plots demonstrating correlations between percent area covered by β-amyloid (Aβ) and thioflavin-S plaques (A), Aβ and butyrylcholinesterase (BuChE) plaques (B), and BuChE and thioflavin-S plaques (C).

**FIGURE 5.** Comparison of the area covered by plaques stained for β-amyloid (Aβ), butyrylcholinesterase (BuChE), and thioflavin-S in representative regions of the 15-month-old APPSwe/PSEN1DE9 mouse brain.
Based on BuChE histochemistry, followed by Aβ immunofluorescence, all BuChE-positive plaques appeared to be Aβ positive, but not all Aβ-positive plaques contained BuChE activity when the 2 photomicrographs of the same section were merged (Fig. 6D–F). This parallels the observation that the area covered by Aβ was higher than that covered by BuChE (Table; Fig. 5).

Based on BuChE histochemistry followed by thioflavin-S staining, some, but not all, BuChE-positive plaques appear to be thioflavin-S positive when the 2 photomicrographs of
the same section were merged (Fig. 6G–I). This is consistent with the observation that the area covered by BuChE is greater than that covered by thioflavin-S (Table; Fig. 5).

DISCUSSION

The transgenic APP<sub>Swe</sub>/PSEN1dE9 (ADTg) mouse model of AD is known to develop plaques in regions of the brain that largely parallel the accumulation of Aβ plaques in human AD (34, 36, 44–49). Here we show that, as in AD brains (19–26), many of the plaques that accumulate in the brain tissue of this ADTg mouse become associated with BuChE activity. Data regarding the relationship of BuChE-associated activity in plaques of AD transgenic mouse models is limited (51). In the ADTg model, the percent area covered by plaques stained for BuChE fell between the percent area stained for Aβ and that stained with thioflavin-S. Because Aβ immunohistochemical staining detects nonfibrillar and fibrillar Aβ, and thioflavin-S detects only the β-pleated fibrillar amyloid characteristic of AD, the association of BuChE with plaques may represent an intermediate stage between nonfibrillar plaques and the fibrillar plaques, which are β-pleated, thioflavin-S positive, and thought to be neurotoxic (13–15).

In contrast to AD, plaques in this ADTg model did not exhibit AChE activity, which is consistent with previous observations in this model (50). One explanation for this apparent lack of AChE activity associated with plaques may be related to using short incubation times (5 minutes) with acetylthiocholine substrate in the histochemical procedure. This limited incubation was necessary to prevent overstaining of the entire tissue, which, by itself, would preclude AChE-positive structures from being identified. Another possibility for the lack of AChE plaque staining may be related to the observation that AChE is normally bound to plaques via association with heparan sulfate proteoglycans (52, 53). In AD brains, heparan sulfate proteoglycans have also been associated with Aβ aggregation (52, 54, 55) and are colocalized with AChE (53). In the ADTg model used here, heparan sulfate proteoglycans are greatly diminished (56) and could contribute to the observed lack of AChE association with the plaques. The absence of plaque-associated AChE in this model might be an advantage for elucidating the possible role of BuChE in plaque development in the absence of cholinesterase activity.

In the present study, we confirmed previous observations that, in this ADTg mouse model, Aβ plaques accumulate in large numbers in distinct regions of the brain that parallel those in AD. Areas typically affected in this model include the cerebral cortex (46, 47), hippocampal formation (34, 36, 45, 47), amygdala (48, 49), and cerebellum (46). As in other mouse models of AD (57–61), this model also exhibited Aβ pathology in olfactory structures. Most importantly, the distribution of plaques in this ADTg mouse model was similar to that seen in human AD where Aβ plaques also accumulate in the cerebral cortex (62–64), hippocampus (65–67), and amygdala (63, 68–70). However, there were a few areas of the ADTg brain where the distribution of Aβ plaques did not match that in AD. For example, in the MOB of this model, most plaques were present in the granular cell layer (Table; Fig. 3A). In contrast, plaques are found in all layers in the MOB of AD (64, 71, 72).

In each region of the ADTg brain analyzed, the percent area covered by plaque staining was the highest for Aβ immunohistochemistry and lowest for thioflavin-S histofluorescence; the percent area covered by BuChE-stained plaques was consistently between these (Table; Fig. 5). One interpretation of this pattern of plaque staining is that BuChE activity in plaques may signal a transition of Aβ from the nonfibrillar to the fibrillar β-pleated sheet configuration typical of AD plaques. Thus, BuChE may play a role in Aβ aggregation, as suggested previously in AD (23, 27). The mechanism of any BuChE participation in this transformation is not clear but may be related to its propensity for protein-protein interaction (73, 74) or its tendency to be influenced by transition metal ions (26), which are also known to affect Aβ aggregation (75). Because BuChE is associated with a number of Aβ plaques, BuChE-positive plaques might correlate with behavioral changes, as well as synaptic and neuronal loss (45, 48, 50, 76).

In summary, the present study demonstrates that the ADTg mouse exhibits BuChE activity in Aβ and thioflavin-S plaques. Further study of a transgenic mouse model could help clarify the involvement of BuChE in AD pathology. Such a model should be amenable to testing for a possible contribution of that enzyme to plaque maturation with the use of specific inhibitors of BuChE to test for the reduction of thioflavin-S-positive plaque load, as previously suggested (77). The AD mouse model should help establish a chronology of Aβ-, BuChE-, and thioflavin-S-positive plaque formation that could be extrapolated to AD and provide insight into where drugs might be the most effective in preventing the plaques from transforming to the “mature” thioflavin-S-positive state. In addition, an ADTg model should permit investigations into whether BuChE represents an appropriate molecular imaging target for early diagnosis and treatment monitoring of AD.

REFERENCES


© 2012 American Association of Neuropathologists, Inc.


