Neuroglial Cholinesterases in the Normal Brain and in Alzheimer's Disease: Relationship to Plaques, Tangles, and Patterns of Selective Vulnerability

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Butyrylcholinesterase (BChE) and an altered form of acetylcholinesterase (AChE) accumulate in the plaques and tangles of Alzheimer's disease (AD). The sources for these plaque- and tangle-bound cholinesterases have not been identified. We now report that AChE and BChE activities with pH preferences and inhibitor selectivities identical to those of plaque- and tangle-bound cholinesterases are found in the astrocytes and oligodendrocytes of control and AD brains. These glial-type cholinesterases are selectively inhibited by indolamines and protease inhibitors. In control brains glial-type cholinesterases appear confined to the intracellular space, whereas in patients with AD they decorate plaques and tangles as well. In control and AD brains AChE-positive glia are distributed throughout the cortical layers and subcortical white matter, whereas BChE-positive glia reach high densities only in the deep cortical layers and white matter. In non-AD control brains, the ratio of BChE to AChE glia was higher in entorhinal and inferotemporal cortex, two regions with a high susceptibility to the pathology of AD, than in primary somatosensory and visual cortex, two areas with a relatively lower susceptibility to the disease process. There were no age-related differences in the density or distribution of cholinesterase-positive glia. In comparison with age-matched control specimens, AD brains had a significantly higher density of BChE glia and a lower density of AChE glia in entorhinal and inferotemporal regions but not in the primary somatosensory or visual areas. These results suggest that glia constitute a likely source for the cholinesterase activity of plaques and tangles and that a high ratio of BChE- to AChE-positive glia may play a permissive or causative role in the neuropathology of AD.

Wright CI, Geula C, Mesulam M-M. Neuroglial cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability. Ann Neurol 1993;34:373–384

Amyloid plaques in the neuropil (NP) and neurofibrillary tangles (NFT) are the major neuropathological hallmarks of Alzheimer's disease (AD). Light and electron microscopic studies have demonstrated that these structures contain histochemically and immunohistochemically definable acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and that these cholinesterases (ChEs) have different histochemical properties from the ChE associated with intact neuronal cell bodies and axons [1–6]. For example, the AChE of NP and NFT is more resistant to traditional ChE inhibitors but much more sensitive to indolamines and some protease inhibitors [7–9], requires more substrate for inhibition [10], and is histochemically more intensely reactive at a lower pH than the AChE of normal neurons and axons [3].

In the normal cerebral cortex AChE is widely distributed in cholinergic axons and in the perikarya of both cholinergic and cholinoceptive neurons. These constituents of cholinergic pathways undergo severe degeneration in AD (see [11] for review). The enzymatic peculiarities of the NP- and NFT-bound AChE suggest that this enzyme is unlikely to constitute the pathological remains of premorbidly AChE-positive axons and perikarya. There are also considerable discrepancies in the distribution of premorbid AChE-rich neuronal cell bodies and AChE-rich tangles. For example, layer II neurons of entorhinal cortex, which are known to be highly vulnerable to forming AChE-rich tangles, express relatively little AChE in normal brains [3, 4]. Furthermore, premorbid neurons of the cerebral cortex do not express sufficient BChE to account for the abundant and widespread BChE activity in NP and NFT. These considerations led us to explore the possibility that the NP- and NFT-bound ChEs may arise from nonneuronal sources. The purpose of this re-

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Received Feb 26, 1993, and in revised form May 5. Accepted for publication May 6, 1993.

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Table 1. Characteristics of Control and Alzheimer's Disease (AD) Cases Used for Cholinesterase Analysis

Diagnosis	Age (yr)	Sex	Autolysis Time (hr)	Hemisphere	Cause of Death
Control	20	M	9	L	Gunshot
Control	35	F	18	R	Respiratory failure
Control	55	F	8	R	Cardiac failure
Control	68	M	24	R	Cardiac arrest
Control	69	F	21	R	Cardiopulmonary failure
Control	71	M	14	L	Cardiac arrest
Control	72	F	7.5	R	Respiratory failure
Control	75	M	12	R	Cardiac failure
Control	76	M	17	L	Cardiac failure
Control	84	F	3.5	R	Cardiac failure
Control	91	M	4	L	Cardiac failure
AD	67	F	12	R	Respiratory failure
AD	77	F	17	R	Respiratory failure
AD	79	F	6	R	Cardiac arrest
AD	82	F	6	R	Cardiac arrest
AD	87	F	12	R	Cardiac failure

port is to describe experiments aimed at investigating whether the astrocytes and oligodendrocytes of the cerebral cortex could be providing a potential source of nonneuronal ChE for the NP and NFT.

Materials and Methods

Tissue Preparation

The observations in this report are based on the following 16 brains (Table 1): 3 younger non-AD brains (20, 35, and 55 years old), 8 aged non-AD brains (68, 69, 71, 72, 75, 76, 84, and 91 years old), and 5 AD brains (67, 77, 79, 82, and 87 years old). Interval from death to autopsy was 24 hours or less in all cases. In our experience, these intervals do not interfere with ChE histochemistry. Coronal slabs 1 to 2 cm in width were cut and immersion-fixed in 4% paraformaldehyde/0.1 M phosphate buffer (pH 7.4) for 24 to 30 hours. After fixation, tissue was taken through gradually increasing concentrations of sucrose (10–40%) in 0.1 M phosphate buffer (pH 7.4). Fixed tissue slabs were then frozen on dry ice and cut by microtome into sections of 40-μm thickness.

Diagnostic Evaluation

Diagnosis of AD was based on clinical history of progressive dementia and on evidence of multifocal, thioflavine S histofluorescent plaque and tangle populations in quantity and distribution satisfying the criteria reviewed by Khachaturian [12]. The non-AD control designation was based on specific clinical historical information about the absence of dementia or preagonal neurological disease and on the absence of plaques and tangles in sufficient quantities for the diagnosis of AD. The brains from several of the aged patients displayed rare tangles in entorhinal cortex and medial temporal cortex, and some plaques in these and other cortical areas, but fewer than required for a diagnosis of AD. Cresyl violet staining was used for identification of cytoarchitectonic boundaries.

Histochemistry

Brain sections were mounted on chrome-alum–treated slides, dried overnight, and rinsed in 100 mM maleate buffer prior to histochemical analysis. Visualization of ChE was achieved by tissue incubation in diluted (10%) Karnovsky–Roots solution followed by metal ion-diaminobenzidine intensification [13, 14]. This Karnovsky–Roots solution contains 100 mM maleic acid (disodium salt), 0.5 mM sodium citrate, 0.3 mM cupric sulfate, and 0.05 mM potassium ferricyanide. Acetylthiocholine iodide (0.366 mM) was used as the substrate to identify AChE activity. The same concentration of butyrylthiocholine iodide was used as the substrate for identification of BChE activity. Incubation times were 2 to 5 hours. Following incubation, sections were rinsed in 100 mM Tris buffer (pH 7.6). Intensification was performed by incubation with 0.008% (wt/vol) cobalt chloride in 100 mM Tris buffer (pH 7.6). Sections were again rinsed with Tris buffer, and development was carried out in 0.5 mg/ml of diaminobenzidine tetrahydrochloride and 0.01% (vol/vol) hydrogen peroxide in the Tris buffer. Finally, sections were rinsed, dehydrated in graded alcohols, cleared in xylenes, and coverslipped under Permount. These techniques are described in detail elsewhere [3, 7].

In experiments investigating the enzymatic properties of the cholinesterases we varied the pH of the maleate buffer and incubation medium between 6.8 and 8.0 using glacial acetic acid, and assessed the response of the enzymes to the inhibitor substances. For this purpose sections were preincubated with selected concentrations of inhibitor for 30 minutes to 1 hour, and the same concentration of inhibitor was added to the histochemical reaction medium for the full incubation period.

For experiments where the anatomical distribution of the ChE-positive glia was examined, the pH of the histochemical medium was in the range of 6.8 to 7.0. In these studies the AChE reaction medium contained 0.1 mM iso-OMPA or ethopropazine to inhibit BChE and other non-AChE ChEs. As previous experiments had shown that the AChE inhibitors do not alter the resultant distribution of the BChE-reaction product, BChE-positive glia were examined both with and without specific AChE inhibitor (e.g., BW284C51) present.

ChE activity was identified by the presence of the brown-

blue reaction product. This reaction product was not obtained when incubations were performed without the addition of the acetylthiocholine or butyrylthiocholine substrates to the histochemical medium.

Evaluation of Glial Cell Density

ChE-positive glia were counted in selected brain regions. In all regions the stained sections were charted with an *X–Y* plotter. All identifiable glia containing specific histochemical reaction product were counted. The following four areas representing different types of cortex, with differential susceptibility to AD pathology, were examined for counting purposes: entorhinal (ENTO), inferior temporal (ITC), primary somatosensory (SI), and primary visual (VI) areas. The first two areas were examined as they are severely and selectively vulnerable to atrophy, tangle formation, and neuritic degeneration in AD. The other two areas were chosen because they are relatively resistant to these pathological alterations of AD.

Glia were identified by their characteristic nuclear appearance and paucity of cytoplasm. The morphology of the nucleolus and nuclear chromatin was used to distinguish neuroglia from small neurons. No attempt was made to differentiate fibrillary astroglia from protoplasmic astroglia or astrocytes from oligodendrocytes even though the difference between the two was easily detected in most cases. Occasional microglial staining was also seen but represented a small minority of the ChE-positive glial population.

At a magnification of 400×, a square ocular grid scored into 10 × 10 equal divisions of 0.01 mm each was placed with one side parallel to the pial surface in a representative sector in the area under investigation. Every other grid in 8 adjacent columns was counted. Each of the 8 columns spanned the entire cortical thickness and was extended 6 grids deep into the adjacent white matter where every other grid was counted as well. The deeper parts of the white matter were not included in this study. The 1 grid that remained just above the plotted white matter boundary and all that were below this line were considered to be in white matter. The others were counted as part of cortex. Counting was done within the entire grid excluding those cells touching the side of the grid, to avoid counting cells twice. The mean number of glia per ocular grid (i.e., per 0.01 mm^2) and the standard deviation was calculated for cortex and white matter separately. Statistics included analysis of variance (ANOVA), normality testing, and Newman–Keuls multiple range testing. Because of the significant differences between cortical and white matter densities of ChE-positive glia, these values were analyzed separately in the statistical tests. A combined value was used to calculate BChE/AChE glial ratios.

Results

Intense ChE reaction product was detected in intact perikarya and axons as well as in plaques and tangles. The perikaryal and axonal enzyme was almost exclusively of the AChE type, whereas plaques and tangles were associated with AChE as well as BChE reactivity. Numerous neuroglia in AD as well as non-AD specimens, with nuclear characteristics of both oligodendroglia and astroglia, displayed specific ChE reaction product predominantly in the nuclear region (Fig 1).

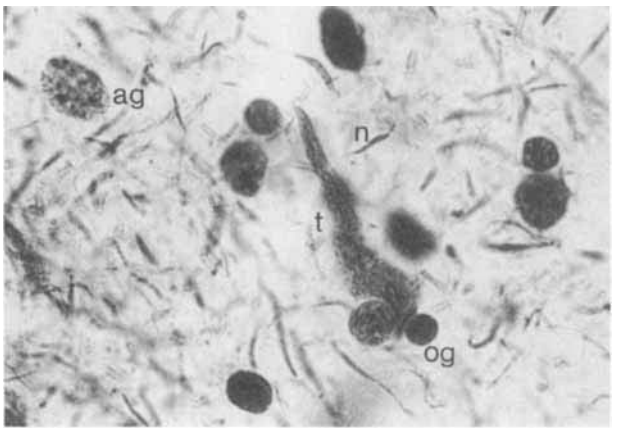

Fig 1. Acetylcholinesterase (AChE) reactivity in tangles (t), *neuropil threads* (n), *astroglia* (ag), *and oligodendroglia* (og) *of entorhinal cortex in the brain of a 67-year-old patient with Alzheimer's disease. The reactivity is specific for AChE as the experiment was performed in the presence of* 10^{-4} *M iso-OMPA, a specific butyrylcholinesterase inhibitor. (× 1,800 before 32% reduction.)*

When acetylthiocholine was used as the substrate in the histochemical solution, glial reaction product was inhibited by up to 0.1 mM of BW284C51 (a specific AChE inhibitor) but not by iso-OMPA (a specific BChE inhibitor). When butyrylthiocholine was used as the substrate, the glial histochemical reaction product was inhibited by 0.1 mM iso-OMPA but not by BW284C51. Thus neuroglia displayed specific AChE and BChE activities.

These specific neuroglial ChE activities were more intensely reactive in histochemical medium of pH 6.8 in comparison with reaction conditions of pH 8.0, which provided more intense staining of normal perikarya and axons. Physostigmine and tacrine were more potent inhibitors of normal perikarya and axonal AChE than glial AChE or BChE (Table 2). Whereas 0.00001 mM concentrations of physostigmine and tacrine inhibited the AChE activity of perikarya and fibers, up to 0.1 mM of these inhibitors was required for inhibition of glial ChE. The plaques and tangles displayed pH preferences and inhibitor responses identical to those of glia.

In contrast to traditional ChE inhibitors, bacitracin and carboxypeptidase inhibitor (CPI; from potato) were more potent inhibitors of glial ChE than of perikaryal and axonal AChE in AD brain and non-AD controls (see Table 2; Fig 2). Bacitracin severely reduced glial AChE at concentrations as low as 0.05 mM. CPI inhibited all glial AChE at 0.05 mM concentrations. Plaque and tangle AChE was similarly inhibited by bacitracin and CPI. Neuroglial BChE was also highly susceptible to these protease inhibitors and was completely inhibited by 0.5 mM concentrations of bacitracin, as was plaque and tangle BChE (see Table 2;

Table 2. Inhibitor Effects of Cholinesterases in Alzheimer's Disease (AD)

Baseline Enzyme Activity (mM)	AChE in Intact Axons and Perikarya	Plaque and Tangle AChE	Plaque and Tangle BChE	Glial AChE	Glial BChE
Physostigmine					
0.00001	−	+++	+++	+++	+++
0.1	−	−	−	−	−
Tacrine					
0.00001	−	+++	+++	+++	+++
0.1	−	−	−	−	−
CPI					
0.05	+++	+	+	−	−
0.1	+++	−	−	−	−
Bacitracin					
0.05	+++	+	−	+	++
0.1	+++	+	−	+	+
0.5	+++	+	−	+	−
1	+++	−	−	+	−
5-HTP					
0.05	+++	++	++	−	−
0.1	+++	+	+	−	−
0.5	+++	−	+	−	−
1	+++	−	−	−	−
5-HT					
0.05	+++	+	+++	−	+++
0.1	+++	−	+++	−	++
0.5	++[a]	−	−	−	−
1	++[a]	−	−	−	−
L-Tryptophan					
0.05	+++	+++	+++	+++	++
0.1	+++	+++	+++	+++	++
0.5	+++	+++	+	++	++
1	+++	++	−	++	+
Tryptamine					
0.05	+++	+++	+++	+++	+++
0.1	+++	+++	+++	+++	+++
0.5	+++	+++	++	++	+++
1	+++	++	+	++	++
Tyrosine					
1	+++	+++	+++	+++	+++
Tyramine					
1	+++	+++	+++	+++	+++
Glycine					
1	+++	+++	+++	+++	+++
Pepstatin A					
1	+++	+++	+++	+++	+++

Comparisons are based on matching tissue sections from the same cytoarchitectonic area and cortical layer in the brains described in Materials and Methods. The examination included the hippocampus, entorhinal cortex, inferotemporal cortex, insula, medial frontal cortex, and the cingulate gyrus. Only the results that were consistently obtained in all specimens investigated for a given variable are included. The results show the various effects of different substances on the visualization of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in glia and the histopathological structures of AD in comparison with perikaryal and fiber AChE.

[a]At these concentrations of 5-hydroxytryptamine (5-HT), a patchy precipitate formed over the sections. In the areas that were free of these precipitates, the AChE staining in intact neuronal cell bodies and axons appeared to be preserved or only very slightly decreased.

+++ = intense baseline staining; ++ = moderate decrease from baseline intensity; + = severe decrease from baseline intensity; (−) = total inhibition of staining.

see Fig 2). The acid protease inhibitor pepstatin A had no effect on either ChE activities in any tissue component at 0.1 mM concentrations (see Table 2).

Selected indolamines also inhibited glial AChE without substantially affecting that of neurons and axons. At concentrations of 0.05 mM, 5-HT and 5-HTP completely inhibited glial AChE (see Table 2; see Fig 2). Tryptophan and tryptamine were less effective inhibitors of glial ChE. The AChE of neuronal cells and axons was not inhibited at any of these indolamine concentrations. Neuroglial AChE was as sensitive, or more sensitive, to the effects of indolamines than plaque and tangle AChE. Glial BChE exhibited similar properties as glial AChE with the exception that glial BChE was more sensitive to L-tryptophan and less sensitive to 5-HT than glial AChE. Formation of the ChE reaction products was unaffected by 1 mM concentrations of tyrosine, tyramine, and glycine. These results indicate that glial ChE has enzymatic properties like that of NP and NFT but different from neuronal AChE.

Lamination

The density of AChE-rich glia was almost equal in the cortex and juxtacortical white matter in ENTO and ITC (Table 3). In the SI and VI areas there was a (nonsignificant) trend toward a higher density of AChE-positive glia in cortex compared with white matter. In contrast, BChE-positive glia in ENTO and ITC were significantly denser ($p < 0.05$) in white matter (see Table 3; Figs 3, 4). The BChE lamination effect was less pronounced in VI and SI. Thus AChE-rich glia are found in all cortical layers and white matter, whereas BChE-containing glia reach high densities only in white matter and deep cortical layers, especially in ENTO and ITC.

Regional Distribution

No significant regional differences were found in AChE glial densities in either the AD or non-AD brains. The density of white matter BChE-positive glia was greater in ENTO than in ITC, but this reached significance ($p < 0.01$) only in AD brains. Furthermore, the density of BChE-rich glia in white matter of ENTO and ITC was significantly greater ($p < 0.01$) than that in SI and VI in both AD and non-AD brains. Non-AD and AD brains showed a higher ratio of BChE/AChE-rich glia in ENTO and ITC than in VI and SI.

Young Versus Old

In areas that we had available (inferotemporal and entorhinal), no statistically significant difference was seen in the density of AChE- and BChE-positive glia (in white matter or cortex) when young and aged non-AD specimens were compared (see Table 3). The ratio of BChE/AChE neuroglia in ENTO and ITC were nearly identical in these two groups. Thus there was no apparent effect of aging in these regions of the temporal lobe.

AD Versus Control

Brains with AD had a significantly higher ($p < 0.01$) density of BChE-rich glia in the white matter of ENTO and ITC when compared with the same areas in age-matched control brains. A similar comparison of SI and VI showed no significant differences. Also, a notable but nonsignificant trend toward reduced concentrations of AChE-positive glia was observed in ENTO and ITC in AD when compared with the non-AD controls. Thus the BChE/AChE glial ratio in entorhinal and inferotemporal areas was higher in AD than in age-matched controls. No such difference occurred in VI or SI.

Neuropathology

We did not quantitate the degree of atrophy or the density of thioflavine S–positive plaques and tangles in the AD brains. However, in keeping with a voluminous literature on this subject [15], a qualitative examination showed that the degree of atrophy and tangle densities were much higher in ENTO and ITC than in SI and VI areas. These latter two areas contained numerous plaques (many of which were nonneuritic) but relatively rare tangles and almost no atrophy.

Discussion

The normal brain displays widespread AChE activity in the axons and cell bodies of cholinergic pathways. This neuronal AChE is greatly reduced in the course of AD [16, 17]. In AD, abundant AChE and BChE activity emerges in plaques and tangles [1–3]. We had reported that the AChE in plaques and tangles had enzymatic properties that differed from those of neuronal AChE and that the amount of plaque- and tangle-bound BChE greatly exceeded the small quantities of neuronal BChE that can be detected in normal brain [4, 7, 8]. These observations suggested that the plaque- and tangle-bound ChEs are likely to have a nonneuronal source. The enzymatic properties that we have identified in the ChEs expressed by neuroglia show that these cells can provide such a nonneuronal source for the ChEs associated with the histopathological structures of AD.

Our experiments also demonstrated the existence of laminar and heretofore unsuspected regional variations in the differential densities of AChE- and BChE-positive neuroglia. We found, for example, that AChE-positive glia are located in all cortical layers and the subcortical white matter, whereas BChE-positive glia reach high densities only in the deepest cortical layer and the subjacent white matter. We had previously shown that AChE-rich tangles were located throughout the cortical laminae, whereas BChE-rich tangles were located only in deeper cortical layers and subjacent

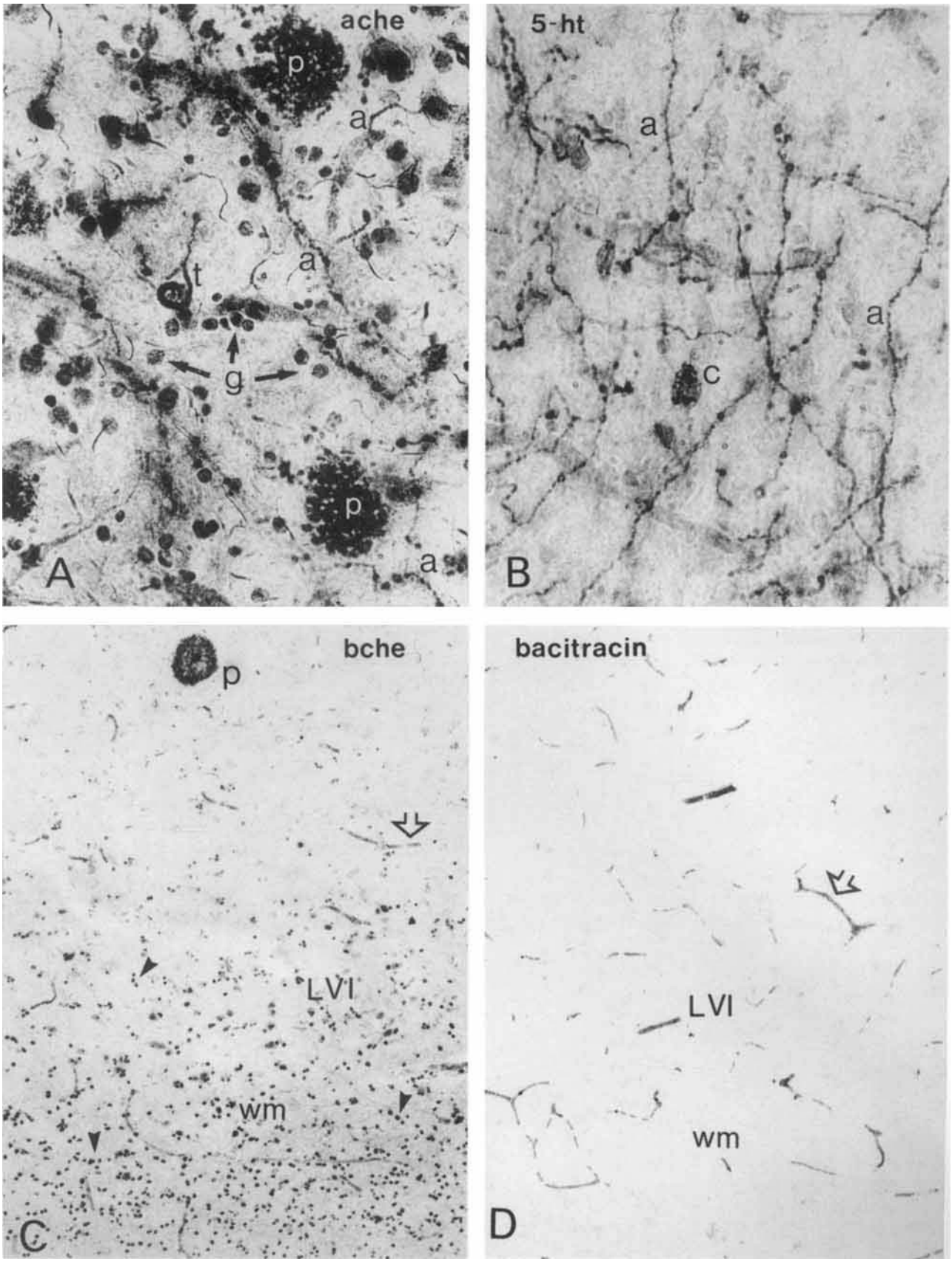
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Table 3. Comparison of Acetylcholinesterase (AChE)- and Butyrylcholinesterase (BChE)-Positive Neuroglia in Alzheimer's Disease (AD) and Control Brain (Aged and Young)

	AChE		BChE		BChE/AChE
	Ctx	Wm	Ctx	Wm	Total (Wm + Ctx)
AD					
ENTO	5.7 ± 3.4	7.4 ± 7.1	1.7 ± 0.97	46.5 ± 12.8	3.7
ITC	2.3 ± 2.3	1.5 ± 1.7	1.2 ± 0.9	30.8 ± 11.7	6.9
SI	8.1 ± 4.2	3.0 ± 1.8	0.77 ± 0.59	4.4 ± 2.7	0.47
VI	9.6 ± 7.5	0.65 ± 0.19	0.7 ± 1	0.34 ± 0.30	0.10
Aged non-AD					
ENTO	8.5 ± 1.5	12.8 ± 3.3	4.6 ± 2.3	16.5 ± 4.9	0.99
ITC	8.6 ± 1.5	9.5 ± 5.8	2.0 ± 1.6	17 ± 6.1	1.0
SI	9.2 ± 2.3	5.3 ± 2.1	2.1 ± 0.9	5.4 ± 3.9	0.52
VI	12.1 ± 0.6	3.7 ± 2.3	1.1 ± 1.3	1.1 ± 0.4	0.13
Young non-AD					
ENTO	8.0 ± 0.5	10.7 ± 1.9	0.59 ± 0.16	16.6 ± 4.7	0.91
ITC	9.5 ± 2.6	15.9 ± 4.3	1.2 ± 0.6	19.0 ± 3.0	0.80

Numerical values are mean neuroglial counts and standard deviations for a tissue area of 0.01 mm^2. All specimens from normal control subjects >65 years old were considered aged, those <65 years of age were considered young.

Ctx = cerebral cortex; Wm = white matter; ENTO = entorhinal area; ITC = inferotemporal area; SI = primary somatosensory area; VI = primary visual area.

white matter [18]. This concordance in laminar distribution supports the inference that glia may constitute the source of the ChE in the histopathological structures of AD.

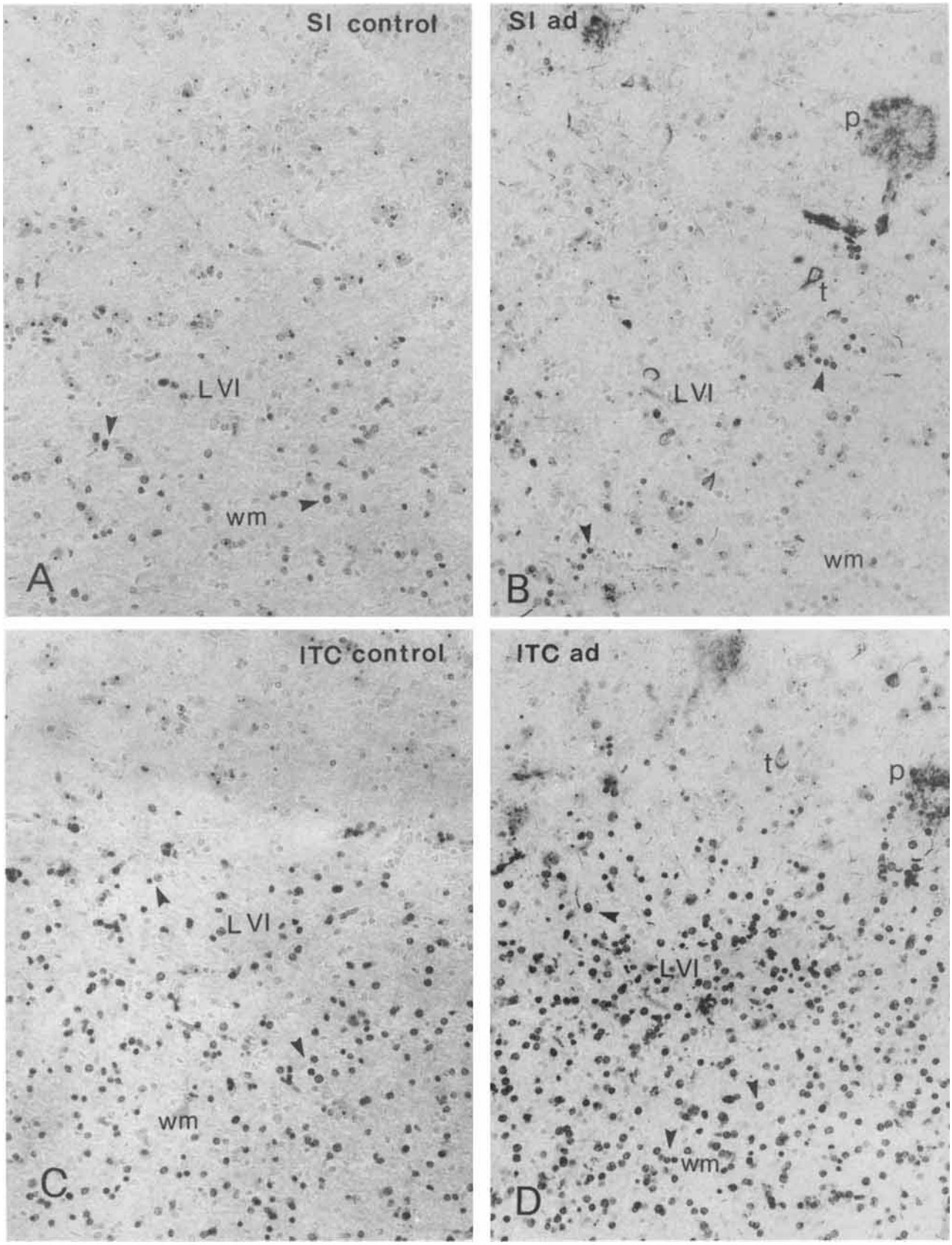
Previous investigators had shown that astrocytes and oligodendroglia express ChE activity. These studies had also reported that BChE-positive glia are most predominant in subcortical white matter, whereas AChE-positive glia are found in all layers of the cerebral cortex [19–21]. Biochemical studies indicate that BChE activity in the human brain is higher in white matter than gray matter of the cerebral cortex [22–24].

Fig 2. Inhibitor sensitivity of cholinesterase-positive neuroglia. (A) Medial frontal area, layer 5 in the brain of an 82-year-old patient with Alzheimer's disease (AD). At a pH of 6.8 acetylcholinesterase (AChE) activity is seen in plaques (p) *and tangles* (t) *and in the few remaining cholinergic axons* (a). *Many neuroglia* (g) *are also AChE positive. (B) Same brain region processed using the same AChE reaction but in the presence of 0.05 mM 5-HT (serotonin). The AChE in plaques, tangles, and glia is almost completely inhibited, but the neuronal AChE in axons* (a) *and neuronal cells* (c) *remains intact. (A, B: × 578 before 13% reduction.) (C) Inferotemporal area from the brain of a 71-year-old control subject. There is intense butyrylcholinesterase (BChE) activity in rare plaques* (p) *and numerous neuroglia* (arrowheads). *Erythrocyte-containing vessels yield a faint but nonspecific reaction* (open arrow). *Note that BChE-positive glia are much denser in layer 6* (LVI) *and in the subcortical white matter* (wm). *(D) Same part of the brain processed with the identical BChE reaction in the presence of 0.5 mM bacitracin. The nonspecific vessel staining persists* (open arrow), *but the plaque- and tangle-associated and glial BChE is completely inhibited.*

The differential lamination of AChE and BChE glia that we observed is consistent with these findings and also leads to the suggestion that most cortical ChE-positive glia express only AChE, whereas the glia in deeper cortical layers and white matter also express BChE. We do not know if this latter group of glia expresses both AChE and BChE or if there are two independent populations, each expressing one of these ChEs.

The suggestion that neuroglia provide a possible source for the plaque- and tangle-associated ChEs is based on the observation that they express ChEs with identical patterns of pH preference and inhibitor selectivity to indolamines and protease inhibitors. The converse possibility that plaques and tangles provide the source for the glial ChEs cannot be supported because non-AD brains, young as well as old, also contain glia with identical ChE enzymatic profiles. The different direction of change displayed by BChE and AChE glia also challenges the possibility that the prominence of ChE-expressing glia could be a secondary and nonspecific outcome of the gliosis associated with AD.

Our experiments in ENTO, ITC, SI, and VI areas indicate that AChE- and BChE-expressing glia are part of the normal adult brain and that the density of these neuroglia does not display major age-related changes. In the normal brain, entorhinal and inferotemporal areas contained a higher density of BChE-positive glia than primary somatosensory or visual areas. When compared with age-matched control brains, the AD brain displayed a further increase in the density of BChE-positive glia and a decrease in the density of AChE-positive glia, but only in the entorhinal and inferotemporal regions. In AD, the entorhinal and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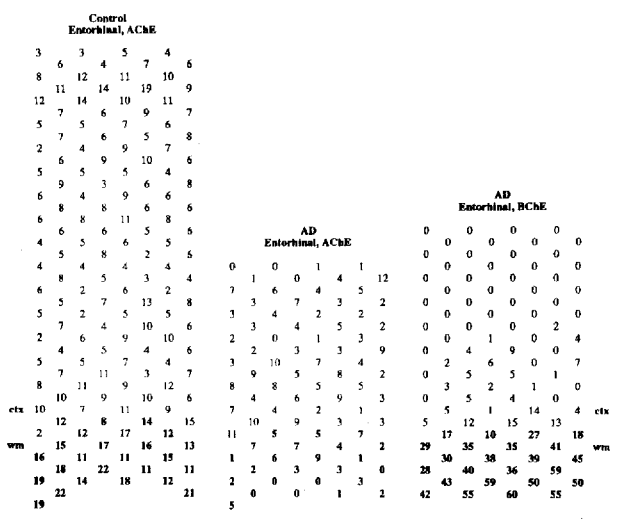

Fig 4. Each number represents the total count of cholinesterase-positive neuroglia in a 10 × 10 reticle of total area 0.01 mm². The center-to-center distance between any two counted fields in a given row was 0.2 mm. Numbers representing glial counts in the white matter are shown in bold. The first row of counts are from the subpial region in layer 1. The counts above the designation cortex are in the gray matter; those below are in the white matter (wm). *Note that the cortical thickness is reduced in Alzheimer's disease (AD). The control subject was 69 years old. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in AD are from matching sections in the same 82-year-old patient.*

Fig 3. Regional and Alzheimer's disease (AD)-related variations in the distribution of butyrylcholinesterase (BChE)-positive neuroglia. The conditions for the BChE histochemical reactions in A through D are identical. (× 296 before 18% reduction.) (A) Primary somatosensory cortex (SI) in a 71-year-old control subject. Few BChE-positive glia (arrowheads) *are seen, mostly in layer 6* (LVI) *and the subcortical white matter* (wm). *(B) The same cytoarchitectonic area in an 82-year-old patient with AD. BChE activity is seen in plaques* (p), *tangles* (t), *and neuroglia* (arrowheads). *A and B have similar densities of neuroglia. (C) Inferotemporal cortex* (ITC) *from the same control subject as in A. There is a higher density of BChE-positive neuroglia* (arrowheads) *than in SI. These neuroglia are also concentrated in layer 6* (LVI) *and white matter* (wm). *(D) Inferotemporal cortex* (ITC) *from the same AD patient as in B. There is BChE reactivity in plaques* (p), *tangles* (t), *and numerous neuroglia* (arrowheads). *The density of BChE-positive neuroglia is higher when compared with SI of the same brain (B) and the same cytoarchitectonic area in the control brain (C).*

inferotemporal areas are known to display earlier and more severe neuropathological changes than primary sensory areas [15]. Our observations therefore suggest that a high density of BChE-positive glia or perhaps a high ratio of BChE/AChE glia may act as a permissive and/or causative factor in the neuropathology of AD.

The molecular basis of the enzymatic distinction between neuronal and glial AChE is poorly understood and may reflect differential splicing or posttranslational modifications. The tetrameric globular form of AChE is predominant in neurons, but AD is associated with a different distribution of molecular forms and perhaps also with the emergence of asymmetrical and monomeric forms in association with plaques and tangles [25–30]. Proteoglycans, which are present in plaques and tangles [31–33] and which are a known glial product [34–37], may be the substance that binds ChE to plaques and tangles [25].

ChEs are phylogenetically ancient and ubiquitous molecules that almost certainly participate in processes other than neurotransmission, including neural differentiation, plasticity, and perhaps the cleavage of peptide bonds. The role of ChEs in protein processing has been claimed by some and denied by others [38–55]. True proteolytic activity is unlikely to be an intrinsic property of the AChE or BChE gene products and probably reflects the activity of substances that may naturally copurify with ChEs [50–55]. However, it is generally accepted that ChEs display an intrinsic aryl–acylamidase activity that can be inhibited by indolamines [43–47]. The existence of the aryl–acylamidase activity together with the esterase activity could give the ChEs the potential to act as proteases because these two activities may be used collectively for protein bond cleavage [48, 49]. Our finding that plaque, tangle, and glial ChEs are selectively vulnerable to indolamines and protease inhibitors suggests that the catalytic sites of these ChEs may be more closely associated with amidase- (or protease)-like properties than the catalytic sites of the ChE found in the majority of normal neurons and their axons [9].

Current hypotheses for the pathogenesis of AD focus on the excessive production, abnormal structure, or aberrant processing of a membrane-spanning β-amyloid precursor protein (βAPP), which contains an amyloidogenic βA4 domain. Normally, the βAPP is processed into various moieties. Some result from cleavage within the βA4 domain and are therefore nonamyloidogenic; others result from alternative cleavage sites that yield the uninterrupted sequence of the βA4 fragment. The βA4 fragment may be released into the extracellular space in a soluble state or may lead to the formation of the insoluble amyloid plaques seen in advanced age and AD [56–58]. The insoluble βA4 deposits may have direct neurotopic effects and may conceivably induce local neuritic degeneration and tan-

gle formation [58]. Alternatively, an aberrant biogenesis and turnover of βAPP may exert a pathogenetic influence through the loss of a poorly understood but crucial function that this protein may have in the normal brain. According to recent observations, for example, βAPP (or some of its nonamyloidogenic-released forms) may bind proteoglycans of the extracellular matrix and may participate in cell adhesion, neurite extension, and protection from neurotoxicity [59–60a]. Such observations raise the possibility that βAPP may act as a neurotrophic growth factor and that its aberrant or deficient function may underlie, at least in part, the pathogenesis of AD.

ChEs may participate in the processing of βAPP and may influence its putative trophic functions. Experiments in chick muscle cultures show that a protease activity that copurifies with AChE can cleave βAPP within the βA4 domain to generate nonamyloidogenic end products [61]. This AChE-associated protease activity has also been shown to release βAPP from extracellular matrix preparations [62]. The specific effects of BChE and of different molecular forms of AChE on βAPP remain unknown.

ChEs may also enter into competitive or complementary interactions with the trophic, growth-promoting, or cell-anchoring functions of βAPP. For example, AChE promotes cell differentiation, neurite extension, and synaptogenesis in a variety of neural systems (see [42] and [63] for discussion). Recent observations also indicate that the plaque- and tangle-associated ChEs bind proteoglycans of the extracellular matrix [25]. Assuming that the plaque and tangle ChEs are derived from glia, glial ChE and βAPP may thus share common binding sites. These two proteins may even share a common origin because recent observations show that astroglia constitute one important source of βAPP and the soluble βA4 fragment [56]. The excessive accumulation of glial ChEs at extracellular binding sites that are also used by βAPP could interfere or compete with the normal function of βAPP. Alternatively, the glial ChEs may be secreted in an attempt to promote the regenerative processes that can no longer be sustained by an aberrant βAPP or that become jeopardized by excessive accumulations of the βA4 fragment.

According to our current hypothesis, glial ChEs represent molecular forms that may participate (either directly or through associated substances) in peptide bond processing and neurotrophic function but that probably have little, if any, role in conventional cholinergic neurotransmission. These glial ChEs, together with other glial markers such as protease nexin 1 [64, 65], α-1-antichymotrypsin [66], and α-1-antitrypsin [66a] may help to regulate the dynamic balance between tissue proteases and their inhibitors and may also influence complex processes related to plasticity, regeneration, and cell adhesion. In the normal brain, glial ChE is detected predominantly in the intracellular space. In AD there may be excess production or secretion, reduced clearance, or increased adhesion to extracellular proteoglycans. The resultant extracellular accumulation of glial ChE may alter the response of the nervous system to stress and injury. This may represent a direct effect of the ChEs or it may be secondary to their interactions with the physiological functions and processing of other molecules such as βAPP. The way in which glial ChEs reach plaques and tangles and their specific role in the genesis of these histopathological structures remains to be elucidated. Our observations also suggest that a high ratio of BChE/AChE glia may act as a marker for brain regions that are selectively vulnerable to the neuropathology of AD. Perhaps only individuals with a premorbidly excessive BChE/AChE ratio are vulnerable to AD or perhaps this ratio further increases in specific regions of the brain as part of the pathogenetic process. The inference that neuroglia contribute to the pathogenesis of AD is in keeping with preliminary observations that show that astrocytes may participate in the production of βA4 and in tangle formation [56, 67].

Large numbers of patients with AD have been given ChE inhibitors to enhance central cholinergic transmission. Based on our current understanding of the multifaceted neuropathology of AD, it is unlikely that enhancement of cholinergic transmission will, by itself, have a substantial therapeutic effect [11]. As shown in Table 2, traditional ChE inhibitors (e.g., physostigmine and tacrine) that have been used in these therapeutic trials are approximately 100 times less effective in inhibiting glial than neuronal ChE. Our observations suggest that other ChE inhibitors that can selectively target glial ChE may offer an alternative and perhaps more effective approach to the treatment and prevention of AD.

We thank Leah Christie, Kristin Bouvé, and Christine Calabrese for their expert secretarial and technical assistance.

This work was supported in part by the Javits Neuroscience Investigator Award NS20285 (M. M. M.), an Alzheimer's Disease Research Center Grant AG05134, a grant from the National Institutes for Aging AG10282 (C. G.), and a research scholarship from the American Federation for Aging Research/Hartford Foundation (C. I. W.), and from the National Medical Fellowships, Inc, Commonwealth/Bristol Meyers-Squibb Fellowship in Academic Medicine (C. I. W.) and a pilot grant from Marion Merrell Dow Corporation.

References

1. Friede RL. Enzyme histochemical studies of senile plaques. J Neuropathol Exp Neurol 1965;24:477–491
2. Perry RH, Blessed G, Perry EK, Tomlinson BE. Histochemical observations on the cholinesterase activities in the brains of elderly normal and demented (Alzheimer-type) patients. Age Aging 1980;9:9–16
3. Mesulam M-M, Moran M. Cholinesterases within neurofibril-

lary tangles of aging and Alzheimer's disease. Ann Neurol 1987;22:223–228
4. Mesulam M-M, Geula C. Shifting patterns of cortical cholinesterases in Alzheimer's disease: implications for treatment, diagnosis, and pathogenesis. Adv Neurol 1990;51:235–240
5. Carson KA, Geula C, Mesulam M-M. Electron microscopic localization of cholinesterase activity in Alzheimer brain tissue. Brain Res 1991;540:204–208
6. Geula C, Brimijoin S, Mesulam M-M. Immunocytochemical detection of cholinesterases in Alzheimer's plaques and tangles. Neurology 1992;41:376
7. Mesulam M-M, Geula C, Moran A. Anatomy of cholinesterase inhibition in Alzheimer's disease: effect of physostigmine and tetrahydroaminoacridine on plaques and tangles. Ann Neurol 1987;22:683–691
8. Geula C, Mesulam M-M. Special properties of cholinesterases in the cerebral cortex of Alzheimer's disease. Brain Res 1989; 498:185–189
9. Wright C, Geula C, Mesulam M-M. Protease inhibitors and indolamines selectively inhibit cholinesterases in the histopathologic structures of Alzheimer's disease. Proc Natl Acad Sci USA 1993;90:683–686
10. Schätz C, Geula C, Mesulam M-M. Competitive substrate inhibition in the histochemistry of cholinesterase activity in Alzheimer's disease. Neurosci Lett 1990;117:56–61
11. Geula C, Mesulam M-M. Cholinergic systems and related neuropathological predilection patterns in Alzheimer's disease: In: Terry R, Katzman R, Bick K, eds. Alzheimer's disease. New York: Raven Press, 1993 (in press)
12. Khachaturian Z. Diagnosis of Alzheimer's disease. Arch Neurol 1985;42:1097–1105
13. Karnovsky MJ, Roots L. A "direct coloring" thiocholine method for cholinesterases. J Histochem Cytochem 1964;12:219–221
14. Tago H, Kimura H, Maeda T. Visualization of detailed acetylcholinesterase fiber and neuron staining in rat brain by a sensitive histochemical procedure. J Histochem Cytochem 1986; 34:1431–1438
15. Kemper T. Neuroanatomical and neuropathological changes in normal aging and in dementia. In: Albert ML, ed. Clinical neurobiology of aging, vol 2. New York: Oxford University Press, 1984:9–52
16. Geula C, Mesulam M-M. Cortical cholinergic fibers in aging and Alzheimer's disease: a morphometric study. Neuroscience 1989;33:469–481
17. Heckers S, Geula C, Mesulam M-M. Acetylcholinesterase-rich pyramidal neurons in Alzheimer's disease. Neurobiol Aging 1992;13:455–460
18. Coleman A, Geula C, Price B, Mesulam M. Differential laminar distribution of acetylcholinesterase and butyrylcholinesterase containing tangles in the cerebral cortex of Alzheimer's disease. Brain Res 1992;596:340–344
19. Roessmann U, Friede R. Changes in butyrylcholinesterase activity in reactive glia. Neurology 1966;16:123–129
20. Friede RL. A comparative histochemical mapping of the distribution of butyrylcholinesterase in the brains of four species of mammals, including man. Acta Anat 1967;66:161–177
21. Silver A. The biology of cholinesterases. New York: American Elsevier Publishing, 1974
22. Ord MG, Thompson RHS. Pseudocholinesterase activity in the central nervous system. J Biochem 1952;51:245–251
23. Lumsden CE. Aspects of the chemistry of the myelin sheath cell complex. In: Williams D, ed. Modern trends in neurology. London: Butterworth, 1957:130–147
24. Lumsden CE. The problem of correlation of quantitative methods and tissue morphology in the central nervous system. The distribution of cholinesterases. In: Richter D, ed. Metabolism of the nervous system. New York: Plenum Press, 1957:91–100
25. Kalaria RN, Kroon SN, Grahovac I, Perry G. Acetylcholinesterase and its association with heparan sulfate proteoglycans in cortical amyloid deposits of Alzheimer's disease. Neuroscience 1992;51:177–184
26. Atack JR, Perry EK, Bonham JR, et al. Molecular forms of acetylcholinesterase in senile dementia of Alzheimer: selective loss of the intermediate (10S) form. Neurosci Lett 1983; 40:199–204
27. Younkin SG, Goodridge B, Katz J, et al. Molecular forms of acetylcholinesterase in Alzheimer's disease. Fed Proc 1986; 45:2982–2988
28. Siek GC, Katz LS, Fishman EB, et al. Molecular forms of acetylcholinesterase in subcortical areas of normal and Alzheimer disease brain. Biol Psychiatry 1990;27:573–580
29. Navaratnam DS, Priddle JD, McDonald B, et al. Anomalous molecular forms of acetylcholinesterase in cerebrospinal fluid in histologically diagnosed Alzheimer's disease. Lancet 1991; 337:447–450
30. Arendt T, Brückner MK, Lange M, Bigl V. Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resembles embryonic development—a study of molecular forms. Neurochem Int 1992;21:381–396
31. Snow AD, Mar H, Nochlin D, et al. The presence of heparan sulfate proteoglycans in the neuritic plaques and congophilic angiopathy in Alzheimer's disease. Am J Pathol 1988;133:456–463
32. Perry G, Siedlak SL, Richey P, et al. Association of heparan sulfate proteoglycans with the neurofibrillary tangles of Alzheimer's disease. J Neurosci 1991;11:3679–3683
33. Snow AD, Mar H, Nochlin D, et al. Peripheral distribution of dermatan sulfate proteoglycans (decorin) in amyloid containing plaques and their presence in neurofibrillary tangles of Alzheimer's disease. J Histochem Cytochem 1992;40:105–113
34. Norling B, Glimelius B, Westermark B, Wasteson A. A chondroitin sulfate proteoglycan from human cultured glial cells aggregates with hyaluronic acid. Biochem Biophys Res Commun 1978;84:914–921
35. Norling B, Glimelius B, Wasteson A. Heparan sulfate proteoglycan of cultured cells: demonstration of a lipid- and matrix-associated form. Biochem Biophys Res Commun 1981;103: 1265–1272
36. Norling B, Glimelius B, Wasteson A. A chondroitin sulfate proteoglycan from human cultured glial and glioma cells. Biochem J 1984;221:845–853
37. Threlkeld A, Adler R, Hewitt AT. Proteoglycan biosynthesis by chick embryo retina glial-like cells. Dev Biol 1989;132:559–568
38. Small DH, Ismael Z, Chubb IW. Acetylcholinesterase exhibits a trypsin-like and metalloexoperoxidase-like activity in cleaving a model peptide. Neuroscience 1987;21:991–995
39. Small DH, Simpson RJ. Acetylcholinesterase undergoes autolysis to generate trypsin-like activity. Neurosci Lett 1988;89:223–228
40. Small DH. Serum acetylcholinesterase possesses trypsin-like and carboxypeptidase B-like activity. Neurosci Lett 1988;95: 307–312
41. Small DH. Acetylcholinesterase: zymogens of neuropeptide processing enzymes? Neuroscience 1989;29:241–249
42. Small DH. Non-cholinergic actions of acetylcholinesterase: proteases regulating cell growth. Trends Biochem Sci 1990;15: 213–216
43. Oomen A, Balasubramanian AS. The inhibition of brain aryl acylamidase by 5-hydroxytryptamine and acetylcholine. Biochem Pharmacol 1977;26:2163–2167
44. George ST, Balasubramanian AS. The identity of the serotonin-sensitive aryl acylamidase with acetylcholinesterase from human erythrocytes, sheep basal ganglia, and electric eel. Eur J Biochem 1980;111:511–524

45. Carroll RT, Hepburn TW, Emmerling MR. Interactions between neuropeptides and acetylcholinesterase. Soc Neurosci Abstr 1991;17:701 (Abstract)
46. Majumdar R, George ST, Balasubramanian AS. Serotonin-sensitive aryl acylamidase activity of platelet acetylcholinesterase. Biochem Pharmacol 1982;31:2319–2325
47. Rao RV, Gnanamuthu C, Balasubramanian AS. Human cerebrospinal fluid acetylcholinesterase and butyrylcholinesterase. Evidence for identity between serum and cerebrospinal fluid butyrylcholinesterase. Clin Chim Acta 1989;183:135–145
48. Balasubramanian AS. Have cholinesterases more than one function? Trends Neurosci 1984:467–468
49. Boopathy R, Balasubramanian AS. A peptidase activity exhibited by human serum pseudocholinesterase. Eur J Biochem 1987;162:191–197
50. Checler F, Vincent J-P. Peptidasic activities associated with acetylcholinesterase are due to contaminating enzymes. J Neurochem 1989;53:924–928
51. Checler F, Grassi J, Masson P, Vincent J-P. Monoclonal antibodies allow precipitation of esterasic but not peptidasic activities associated with butyrylcholinesterase. J Neurochem 1990;55: 750–755
52. Araki W, Nakamura S, Tanaka S, et al. Separation of protease activity from acetylcholinesterase of the electric eel. Neurochem Int 1991;19:537–541
53. Carroll RT, Emmerling MR. Identification of the trypsin-like activity in commercial preparations of eel acetylcholinesterase. Biochem Biophys Res Commun 1991;181:858–862
54. Carroll RT, Emmerling MR. Trypsin activity is not intrinsic to acetylcholinesterase. Soc Neurosci Abstr 1992;18:1246 (Abstract)
55. Appleyard ME. Secreted acetylcholinesterase: non-classical aspects of a classical enzyme. Trends Neurosci 1992;15:485–490
56. Busciglio J, Gabuzda DH, Matsudaira P, Yankner BA. Generation of β-amyloid in the secretory pathway in neuronal and non-neuronal cells. Proc Natl Acad Sci USA 1993 (in press)
57. Selkoe D. The molecular pathology of Alzheimer's disease. Neuron 1991;6:487–498
58. Yankner B, Mesulam M-M. β-Amyloid and the pathogenesis of Alzheimer's disease. N Engl J Med 1991;325:1849–1857
59. Roch J-M, Jin L-W, Ninomiya H, et al. Biologically active domain of the secreted form of the β/A4 protein precursor. In: Nitsch RM, Growdon JH, Corkin S, Wurtman RM, eds. Alzheimer's disease: amyloid precursor proteins, signal transduction, and neuronal transplantation. Cambridge, MA: Center for Brain Sciences and Metabolism Charitable Trust, 1993:169–177
60. Robakis NK, Vassilacopoulou D, Efthimiopoulos S, et al. Cellular processing and proteoglycan nature of amyloid precursor proteins. In: Nitsch RM, Growdon JH, Corkin S, Wurtman RM, eds. Alzheimer's disease: amyloid precursor proteins, signal transduction, and neuronal transplantation. Cambridge, MA: Center for Brain Sciences and Metabolism Charitable Trust, 1993:161–168
60a. Mattson MP, Cheng B, Culwell AR, et al. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the β-amyloid precursor protein. Neuron 1993;10:243–254
61. Small DH, Moir RD, Fuller SJ, et al. A protease activity associated with acetylcholinesterase releases the membrane bound form of the amyloid precursor of Alzheimer's disease. Biochemistry 1991;30:10795–10799
62. Small DH, Nurcombe V, Michaelson S, et al. Association and release of the amyloid precursor protein of Alzheimer's disease from chick brain extracellular matrix. J Neurosci 1992;12: 4143–4150
63. Mesulam M-M, Geula C. Acetylcholinesterase-rich neurons of the human cerebral cortex: cytoarchitectonic and ontogenetic patterns of distribution. J Comp Neurol 1991;306:193–220
64. Guenther J, Nick H, Monard D. A glia derived neurite-promoting factor with protease inhibiting activity. EMBO J 1985; 4:1963–1966
65. Stone RS, Nick H, Hofsteenge J, Monard D. Glia-derived neurite-promoting factor is a slow binding inhibitor of trypsin, thrombin and urokinase. Arch Biochem Biophys 1989;252: 237–244
66. Miyakawa T, Katsuragi S, Yamashita K, et al. The distribution of alpha 1-antichymotrypsin and amyloid production in the brain in Alzheimer's disease. Virchows Arch B Cell Pathol 1992;61: 331–335
66a. Gollin PA, Kalaria RN, Eikelenboom P, et al. Alpha-1-antitrypsin and alpha-1-antichymotrypsin are in the lesions of Alzheimer's disease. Neuroreport 1992;3:201–203
67. Ikeda K, Akiyama H, Haga C, Haga S. Evidence that neurofibrillary tangles undergo glial modification. Acta Neuropathol 1992;85:101–104