Soluble and Membrane-Bound Forms of Brain Acetylcholinesterase in Alzheimer’s Disease

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SCHEGG, K. M., L. S. HARRINGTON, S. NEILSEN, R. M. ZWEIG AND J. H. PEACOCK. Soluble and membrane-bound forms of brain acetylcholinesterase in Alzheimer’s disease. NEUROBIOl AGING 13(6) 697–704, 1992.—In order to determine the effect of Alzheimer’s disease on the relative distribution of soluble and membrane-bound molecular forms of acetylcholinesterase (AChE) in the brain, postmortem samples (delay interval less than 12 h) were obtained from parietal cortex (Brodmann area 40) and hippocampus as well as the areas containing their respective projection nuclei, i.e., substantia innomina and septal nucleus, in 9 patients with Alzheimer’s disease (AD) and 4 normal controls. The monomer (G1), dimer (G2), and tetramer (G4) forms of AChE were examined. In AD compared to controls, significant changes occurred in area 40 and hippocampus but not in the areas containing projection nuclei, and included loss of mean total AChE activity, decrease in the relative percentage of membrane-bound G4, and increase in the relative percentage of soluble G1–G2. Percent of soluble G4 was unaffected in AD brain. In area 40 but not hippocampus a large increase in percent membrane-bound G1–G2 occurred. Thus, these results emphasize that the selective decrease in membrane-bound G4 accounts for the decrease in total G4 activity in AD brain.

THE role of acetylcholinesterase (AChE) in termination of cholinergic neurotransmission is well recognized, but less well appreciated is the considerable complexity of AChE molecular forms and their biological roles and interrelationships. In the central nervous system (CNS) three globular forms of the enzyme predominate: a monomer (G1; 4–5S), dimer (G2; 6–7S), and tetramer (G4; 10–11S) (30,32,33). Each of these forms may be freely soluble or tightly bound within membranes (5,30,32). Freely soluble forms exist within the cell and are secreted from it (19,37,39). Bound AChE, which requires detergent for solubilization, may be attached to membranes within the cell or be integral to the exterior plasma membrane (13,21). Additionally, in the peripheral nervous system (32) and to a small extent in the CNS (5,33), there exist asymmetric forms of AChE characterized by attachment to collagen-like tails; these forms require high salt concentrations in order to be solubilized.

The relative proportions of the molecular forms of AChE change in relationship to developmental stages of mammalian brain (30,31,34,43) and reflect the function of various brain regions. In human brain, high proportions of G4 (5,14) correlate with areas of high cholinergic activity, as measured by the local activity of choline acetyltransferase (ChAT). Membrane-bound G4 (30) appears to be the physiologically critical form of AChE in cholinergic neurons of the CNS. By contrast, secreted soluble G4 may participate in important noncholinergic functions, because the release of soluble G4 from cerebellum (1) and the dendrites of dopaminergic neurons of substantia nigra (19) is increased by stimulation of these neurons in a manner that is unrelated to cholinergic neurotransmission.

In Alzheimer’s disease (AD), the cerebral cortex and hippocampus display marked decreases in the activities of ChAT and AChE (2,20,22,41) indicating dysfunction of the cholinergic projection system originating in the basal nuclei (medial septal nucleus, nucleus of the diagonal band of Broca, and the nucleus basalis of Meynert). In brains of AD patients, Brodmann area 40 of parietal cortex (6,14) as well as other cortical areas (14,41) and amygdala (14) contain AChE whose relative percentage of G4 is significantly decreased compared to that of control brains. Because it is only membrane-bound G4 and not its other forms that appears to have a critical function in cholinergic neurotransmission, it is important to know if this de-
crease in percent total G1 actually results from a specific decrease in percent membrane-bound G1. Postmortem findings in cortical area 21 from AD patients suggest that the decrease in percent G1 is likely to be caused by a loss of membrane-bound G1 (41). However, the effects of AD on the membrane-bound form of G1 or on the other bound and soluble AChE forms have not been investigated in the septo-hippocampal pathway or in portions of cortex other than area 21. In view of recent clinical trials with AChE inhibitors such as tetrahydraminoacridine (40) and others (3) for symptomatic treatment of AD, it seemed essential to learn as much as possible about the state of AChE in affected areas of the brain in AD.

To this end, we have undertaken analysis of AChE, differentiated into soluble and membrane-bound forms, in extracts of cortical area 40, substantia innominata (containing nucleus basalis of Meynert), hippocampus, and the septal region (containing medial septal nucleus) from AD and control brains.

METHOD

Materials

Triton X-100 and [3H]-acetylcholine iodide (50–100 mCi/mmol) were supplied by NEN Research Products (Boston, MA). [3H]-Acetyl coenzyme A (51–59 mCi/mmol) was obtained from Amersham (Arlington Heights, IL) or from NEN. Acetonitrile and Kalignost were products of Aldrich (Milwaukee, WI). All other biochemicals were from Sigma Chemical Company (St. Louis, MO).

Patients and Brain Areas Sampled

Brain samples from a total of 13 patients were examined in this study. Samples were not obtained from all brain regions in each patient. In 9 of the patients there was a history of dementia and the diagnosis of AD was pathologically confirmed according to the criteria of Khachaturian (24) by counting 15 or more senile plaques per low power microscopic field (×200) of neocortex after visualization by silver staining. The AD patients included 5 females and 4 males ranging in age from 74–84 years (mean age: 78 ± 3 years); autopsy delays ranged from 2% to 11 h. Four control patients, 1 female, and 3 males, were between 65–75 years (mean age 70 ± 4 years); autopsy delays ranged from 3% to 12 h.

Brain areas analyzed included: area 40 (8 AD, 4 controls); substantia innominata (3 AD, 3 controls); hippocampus (8 AD, 4 controls); and septal nucleus (5 AD, 4 controls).

Differential Extraction of Acetylcholinesterase

Samples of Brodmann area 40, substantia innominata, and hippocampus all on the left side of the brain, as well as septal nucleus were removed at autopsy within 12 h of the time of death. The locations and sizes of the samples removed are described in the legend for Fig. 1. The AChE contents were extracted as described in detail previously (38). Briefly, immediately after removal, original homogenates were made by sonicating gently and then treated so as to produce 3 different mixtures: a) a total AChE homogenate was produced by adding an equal volume of 50 mM sodium phosphate, 20 mM EDTA, 2.0 M NaCl, 1.0% Triton X-100, pH 7.4 to an aliquot of the original homogenate; b) an extractable AChE supernatant was made by placing the total AChE homogenate on ice for 30 min, centrifuging at ×48,000 g, for 10 min and collecting the supernatant; and c) a soluble AChE supernatant was produced by centrifuging a portion of the original homogenate at ×48,000 g, for 10 min and collecting the supernatant. The supernatant containing extractable AChE was used for assay of ChAT activity. AChE was determined in the total AChE homogenate and the extractable and soluble AChE supernatants. The supernatants containing soluble AChE and extractable AChE were centrifuged on sucrose gradients to separate AChE molecular forms.

Acetylcholinesterase Assays

AChE was assayed by a modification (37) of the radiometric method of Johnson and Russell (22). Before assay, aliquots of soluble AChE supernatants were diluted with an equal volume of 50 mM sodium phosphate, 20 mM EDTA, 2.0 M NaCl, 1.0% Triton X-100, pH 7.4; other samples were not diluted. Appropriately diluted samples (10 µl) were added to 85 µl of 50 mM Tris, 0.1 M NaCl, 50 mM MgCl2, pH 7.5. Assays were carried out in 7 ml scintillation vials and were initiated by adding 5 µl of 0.01 M [3H]-acetylcholine (12.5 µCi/ml) to samples and incubating at room temperature. Each assay contained 10 "M tetraisopropyl pyrophosphoramide (iso-OMPA) in order to inhibit pseudocholinesterase activity. Assays were stopped and counted as described by Johnson and Russell (22). Previous studies (38) showed that assays were linear with regard to time for at least 2 h and with regard to activity up to approximately 0.017 nmol of total substrate hydrolyzed. Specific activity of AChE was calculated as nmol/min per mg protein.

Choline Acetyltransferase Assays

ChAT activity was determined by a modification of the method of Fonnun (15). A bulk assay mixture was prepared by combining equal quantities of the following solutions: a) 48 mM choline chloride, 60 mM tetrasodium EDTA, 0.15 M sodium phosphate, 6 mg/ml bovine serum albumin, pH 7.4; b) 0.6 mM physostigmine, and c) 13.2 µCi/ml [3H]-acetyl coenzyme A (AcCoA). Solution 3 was prepared just before use by combining [3H]-AcCoA with an unlabeled solution of AcCoA in water such that the resulting AcCoA concentration was 2.4 µM. Ten microliters of assay mixture was pipetted into a 7 ml scintillation vial; the vial was capped, placed in a test tube rack that held the vial tightly, and incubated in a 37° water bath. Assays were initiated by the addition of 10 µl aliquots of samples; all samples were contained in 50 mM sodium phosphate, 20 mM EDTA, 1.0 M NaCl, 0.5% Triton X-100, pH 7.4. Controls contained 0.8 units purified ChAT.

Assays were stopped exactly 1 h after initiation by the addition of 1.73 ml of an ice-cold mixture of 1 part 5 mg/ml Kalignost (tetraphenylboron sodium) in acetonitrile to 2.46 parts 10 mM sodium phosphate, pH 7.4. Immediately, 2.5 ml scintillation fluid (0.05% 2,5-diphenyloxazole and 0.02% 1,4 bis [2-(5-phenyloxazolyl)] benzene in toluene) were added and the vial was rotated in an upright position for 5 s. Assays performed in this manner were linear with regard to time for 1 h and with regard to activity up to approximately 1.8 nmol acetylcholine produced. Specific activity of ChAT was calculated as nmol/min per mg protein.
FIG. 1. Photographs of brain areas from a formalin-fixed brain not used in this study but which are representative of those regions where samples were removed at autopsy for neurochemical analyses. Areas dissected and removed are outlined and indicated by asterisks. Crosby's textbook *Correlative Anatomy of the Nervous System* (10) was used to guide dissections. (A) Septal nucleus: sections taken were located immediately adjacent to the inferior portion of the septum pellucidum (SP; included in the dissection), anterior to the fornix (F), superior to the anterior commissure, and posterior to the rostrom of the corpus callosum (CC). (B) Hippocampus: the area dissected include the dentate and hippocampal gyri, excluding the alveus and fimbria (FIM). Portions of the prosubiculum (adjacent to the subiculum) may have been included in some of the dissections. SUB = subiculum; LGB = lateral geniculate body. (C) Substantia innominata: the samples from this portion of basal forebrain (containing cholinergic neurons of the nucleus basalis of Meynert) were located inferior to the globus pallidus (GP), inferior and medial to the lateral limb of the anterior commissure (AC), and lateral to the optic tract (OT) and supraoptic nucleus. (D) Area 40: a thin section perpendicular to the gyral surface was dissected out from the inferior part of the anterior bank of the supramarginal gyrus (SMG). In order to trim the sample, the underlying white matter was cut away and discarded. PoCG = post central gyrus; STG = superior temporal gyrus.
Sucrose Density Gradient Sedimentation

Supernatants of up to 0.40 ml were centrifuged on 5-25.2% (w/w) exponential sucrose density gradients (26, 27) prepared in 50 mM Tris, 0.2 mM EDTA, 1 M NaCl, 0.5% Triton X-100, 1.05 × 10⁻³ M iso-OMP, pH 7.4. Catalase (11.4S; 820 units) and β-galactosidase (16S; 15 units) were applied to each gradient as standards. Centrifugation was carried out at 4 °C for 41,000 rpm, in a Sorvall TH-641 rotor to a radi/height of 1.12-1.20 × 10⁻¹² (Sorvall OTD 75B ultracentrifuge). Fractions were collected at 4 °C using a Haake-Buchler LC200 fraction collector and assayed for AChE, catalase, and β-galactosidase activities. Peaks from sucrose gradient profiles were cut out of graph paper and each was weighed to determine its contribution relative to the total area under the peaks. Subtraction of the activity contained in the soluble AChE peaks from that contained in the extractable AChE peaks yielded the AChE that was initially bound to membranes.

Protein Assays

Protein content of extractable AChE supernatants was determined by the method of Lowry (25).

Statistical Analyses

Data are reported as mean ± SD. When a single parameter was compared in AD versus control groups, Student's t tests were used to determine significant differences. When more than two groups were compared, analysis of variance (ANOVA) was utilized. Both analyses were performed with programs supplied with Systat (Systat, Inc., Evanston, IL). P values less than 0.05 were considered significant.

RESULTS

Specific Activities of Choline Acetyltransferase and Acetylcholinesterase in Brain Extracts

Extractable AChE supernatants were used for determination of AChE and ChAT specific activities; these supernatants contained both membrane-bound and freely soluble forms of AChE and ChAT. Results are shown in Table 1. For each of the four brain regions, the mean ChAT specific activity in extracts derived from AD patients was less than in extracts derived from control patients and, for area 40 and hippocampus, none of the individual ChAT specific activity values of the AD cases was above the lowest control ChAT specific activity. Differences in ChAT specific activity between AD and control groups were significant for area 40, hippocampus, and septal nucleus but not for substantia innominata.

The mean AChE specific activity in AD extracts was significantly lower than in control extracts for area 40 and hippocampus but not for either of the nuclei. Individual AChE specific activities in AD brain regions, however, covered a wide range. Four out of eight area 40 extracts, 1 out of 3 substantia innominata extracts, 7 out of 8 hippocampal extracts, and 1 out of 5 septal nucleus extracts had AChE specific activities below any in the corresponding control group. However, for all AD brain regions, there were individual extracts which contained AChE specific activities that easily fell within control ranges despite ChAT specific activities well below those of any controls.

Extractability of AD and Control AChE

The potential for AChE to be fully extracted by buffer containing high salt and detergent was checked for each brain region sampled by comparing the AChE activity in each extractable AChE supernatant with that contained in an aliquot of the uncentrifuged homogenate from which the supernatant was derived (i.e., the corresponding total AChE homogenate). Results are shown in Table 1. Nearly 100% of all AChE activity was extracted from hippocampus, substantia innominata, and septal nucleus of control brains. By contrast, although the AChE in area 40 from one control patient was 100% extractable, the area 40 AChE from the remaining 3 control brains was only extracted 85%-91%. The AChE from some of the AD brain regions, particularly from area 40 and hippocampus, was marked by high percentages that could not be extracted. Five of the eight area 40 samples from AD brains contained between 22% and 31% of total AChE activity that was nonextractable. The 5 extracts were those with the lowest ChAT and AChE specific activities.

Analysis of Acetylcholinesterase Molecular Forms

Samples used in this study were initially homogenized gently in low salt buffer before storage at -80 °C. This homogenization was necessary because the small brain region samples used could not be thawed without severe degeneration of tissue. It has been reported that freezing and subsequent thawing of tissue homogenates causes changes in the relative proportions of AChE molecular forms (5). However, in these previous studies the homogenates that were frozen and thawed contained detergent and high salt concentrations. Our testing showed that freezing and thawing of homogenates of mouse

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Patient Type</th>
<th>n</th>
<th>ChAT Specific Activity</th>
<th>AChE Specific Activity</th>
<th>Percent AChE Extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 40</td>
<td>AD</td>
<td>8</td>
<td>0.019 ± 0.015$</td>
<td>1.73 ± 0.83*</td>
<td>79.8 ± 10.8</td>
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<tr>
<td>Area 40</td>
<td>Control</td>
<td>4</td>
<td>0.090 ± 0.012</td>
<td>3.21 ± 1.25</td>
<td>90.7 ± 6.5</td>
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<tr>
<td>Sub. Innominata</td>
<td>AD</td>
<td>3</td>
<td>0.321 ± 0.206</td>
<td>56.77 ± 11.62</td>
<td>92.3 ± 1.3*</td>
</tr>
<tr>
<td>Sub. Innominata</td>
<td>Control</td>
<td>3</td>
<td>0.718 ± 0.469</td>
<td>73.99 ± 25.52</td>
<td>103.0 ± 5.4</td>
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<tr>
<td>Hippocampus</td>
<td>AD</td>
<td>8</td>
<td>0.061 ± 0.034$</td>
<td>6.15 ± 3.03$</td>
<td>92.1 ± 5.5</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Control</td>
<td>4</td>
<td>0.260 ± 0.121</td>
<td>14.43 ± 5.13</td>
<td>99.2 ± 5.3</td>
</tr>
<tr>
<td>Septal Nucleus</td>
<td>AD</td>
<td>5</td>
<td>0.173 ± 0.067*</td>
<td>20.81 ± 8.37</td>
<td>97.5 ± 3.8</td>
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<tr>
<td>Septal Nucleus</td>
<td>Control</td>
<td>4</td>
<td>0.358 ± 0.107</td>
<td>20.19 ± 5.74</td>
<td>99.1 ± 2.2</td>
</tr>
</tbody>
</table>

Units of specific activity are nmol/min per mg protein. *+‡$Indicate that AD samples were significantly different than controls at: *p ≤ 0.05; ‡p ≤ 0.01; $p ≤ 0.001. Sub. Innominata = substantia innominata.
hippocampus and septum prepared in LS buffer produced no changes in molecular form ratios (unpublished data).

The AChE in both soluble and extractable AChE supernatants was analyzed for molecular forms by sucrose density gradient centrifugation; examples of sucrose gradient patterns are shown in Fig. 2. The monomer (G1, \( s_{avg} = 5.0 \pm 0.4 \)), dimer (G2, \( s_{avg} = 7.2 \pm 0.4 \)) and tetramer (G4, \( s_{avg} = 10.9 \pm 0.5 \)) forms of AChE appeared on all sucrose density gradient patterns. There were no differences in the \( S \) value of each molecular form derived from extracts of AD versus control patients. On some of the sucrose gradient patterns, particularly those derived from control brain regions, it was difficult to accurately decipher the boundaries of G1 and G2. Therefore, the percentages of G1 and G2 are presented as a pooled G1-G2 group. Distinct peaks appeared frequently on sucrose gradients from all brain regions of control and AD brains at 8.5S (\( s_{avg} = 8.5 \pm 0.3 \)) and occasionally at \( S \) values around 13-14S and 16-18S. Preliminary attempts to understand the nature of these peaks by sequential extraction experiments suggested that the 8.5S form is globular while the other forms are asymmetric. For calculations of the relative percentages of molecular forms in this study, only the 5-7S (G1-G2) and the 11S (G4) peaks were considered. The sum of these activities was designated as 100%.

Table 2 shows the relative percentages of extractable AChE represented by soluble G1-G2, bound G1-G2, soluble G4, and bound G4 for the brain regions of AD and control patient
groups. In control brains, the average relative percentages of the AChE forms were remarkably similar among the four brain regions. ANOVA indicated that area 40, substantia innominata, hippocampus, and septal nucleus from control brains were not statistically different in terms of the relative percentages of the various molecular forms. The only exception was a significant difference in percent of extractable AChE present in the soluble G±-G2 forms in substantia innominata versus septal nucleus.

Molecular form analysis of brain regions from Alzheimer's patients demonstrated definite changes in the relative percentages of the various AChE forms in some of the regions tested when compared to controls. Overall, patients with the lowest AChE specific activities in any particular brain region exhibited the greatest shifts in relative percentages of molecular forms. The AD patients whose AChE specific activities fell within the range of those seen in controls had small or no shifts in percentages of molecular forms. Changes in molecular form ratios were most pronounced in area 40. In this region, the relative percentages of soluble G±-G2 and of bound G±-G2 increased significantly while the relative percentage of membrane-bound G4 decreased significantly, compared to controls. In substantia innominata and septal nucleus, the decrease in percent bound G4 were significant. The decrease in percent bound G4 of the AChE in AD brain regions could be completely extracted by buffer containing high salt concentration and detergent, the AChE in AD brain regions could not. Most notable was area 40 where as much as 31% of the total AChE was nonextractable. The nonextractable AChE may be bound within structures associated with AD, i.e., neurofibrillary tangles and amyloid plaques. Substantial amounts of acetylcholinesterase specific activity have been reported to be associated with these structures (16,28); the properties of AChE associated with plaques and tangles are different than those of cellular AChE in terms of optimum pH and inhibitor specificity (18).

The purpose of this study was to compare the soluble and membrane-bound molecular forms of AChE in AD and control brains from two cholinergic projection fields which are severely affected in AD, i.e., parietal cortex (Brodmann area 40) and hippocampus. In particular we wanted to examine if there was a change in the membrane-bound G4 form. The analysis was extended to the regions containing projection nuclei, i.e., substantia innominata (containing nucleus basalis of Meynert) and the septal region (containing the medial septal nucleus) in order to compare the projection nuclei and the terminal fields. Initially, the specific activities of ChAT and AChE were analyzed in all brain samples in order to quantitate the change in cholinergic activity. As found in numerous studies (2,12,23), ChAT specific activity was significantly decreased in area 40, hippocampus, and septal nucleus from AD patients. Acetylcholinesterase specific activity was significantly decreased in area 40 and hippocampus. The extractability of AChE in AD brain regions changed in comparison to controls. While the AChE in substantia innominata, hippocampus, and septal nucleus from control brains could be completely extracted by buffer containing high salt concentration and detergent, the AChE in AD brain regions could not. Most notable was area 40 where as much as 31% of the total AChE was nonextractable. The nonextractable AChE may be bound within structures associated with AD, i.e., neurofibrillary tangles and amyloid plaques. Substantial amounts of acetylcholinesterase specific activity have been reported to be associated with these structures (16,28); the properties of AChE associated with plaques and tangles are different than those of cellular AChE in terms of optimum pH and inhibitor specificity (18).

The data in Table 2 indicate that the decreases in percent of total G4 in AD brains are due entirely to a decrease in the percent of the membrane-bound form of G4.

### TABLE 2

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Patient Type</th>
<th>n</th>
<th>Soluble G1-G2</th>
<th>Bound G1-G2</th>
<th>Soluble G4</th>
<th>Bound G4</th>
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<tbody>
<tr>
<td>Area 40 AD</td>
<td>8</td>
<td>12.7 ± 6.1*</td>
<td>30.8 ± 10.1†</td>
<td>13.5 ± 6.1</td>
<td>43.1 ± 10.8‡</td>
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<tr>
<td>Area 40 Control</td>
<td>4</td>
<td>5.3 ± 2.9</td>
<td>9.7 ± 6.0</td>
<td>11.8 ± 3.5</td>
<td>73.2 ± 4.7</td>
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</tr>
<tr>
<td>Sub. Innominata AD</td>
<td>3</td>
<td>1.9 ± 0.7</td>
<td>13.1 ± 6.6</td>
<td>8.8 ± 1.0</td>
<td>76.2 ± 6.7</td>
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</tr>
<tr>
<td>Sub. Innominata Control</td>
<td>3</td>
<td>1.6 ± 0.2</td>
<td>8.1 ± 2.5</td>
<td>9.4 ± 8.6</td>
<td>80.9 ± 7.0</td>
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</tr>
<tr>
<td>Hippocampus AD</td>
<td>8</td>
<td>5.9 ± 2.6*</td>
<td>12.6 ± 7.7</td>
<td>17.0 ± 5.8</td>
<td>64.5 ± 6.5†</td>
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</tr>
<tr>
<td>Hippocampus Control</td>
<td>4</td>
<td>1.9 ± 0.7</td>
<td>7.8 ± 2.0</td>
<td>12.2 ± 3.3</td>
<td>78.1 ± 3.5</td>
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</tr>
<tr>
<td>Septal Nucleus AD</td>
<td>5</td>
<td>3.5 ± 1.3</td>
<td>9.3 ± 3.0</td>
<td>17.8 ± 4.2</td>
<td>69.3 ± 6.9</td>
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<tr>
<td>Septal Nucleus Control</td>
<td>4</td>
<td>3.3 ± 0.9</td>
<td>6.3 ± 2.0</td>
<td>15.2 ± 4.7</td>
<td>75.2 ± 6.7</td>
<td></td>
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</table>

†‡| Indicate that Alzheimer's group was statistically different from controls at: *p ≤ 0.05; †p ≤ 0.01; ‡p ≤ 0.001. The sums of all molecular forms shown in this table were set at 100% for each brain sample. Sub. Innominata = substantia innominata.
soluble G1–G2 (both area 40 and hippocampus) and bound G1–G2 (area 40 only). Changes in AChE in the areas of cholinergic origin, i.e., substantia innominata and septal nucleus, were insignificant. Our results for area 40 are similar to AD-associated changes in molecular forms detected in area 21 (41). In this area, AD patients contained a significantly decreased relative percentage of bound G2 with an increase in the percentage of bound G4. However, in contrast to area 40, no change was detected in the percent soluble G2. Our results for hippocampus are in striking agreement with those obtained from model studies with mice receiving complete lesions of the fornix where thirteen to twenty days after lesioning, the relative percentage of membrane-bound G4 in mouse hippocampus decreased from 77% to 53% (38). In this work, percent soluble G1–G2 increased markedly and percentages of bound G2–G2 and soluble G2 increased moderately.

The data presented here for area 40 and hippocampus seem conclusive; however, the data for the projection nuclei should be considered as suggestive. While hippocampus and area 40 can be specifically and reproducibly dissected, their projection nuclei are small and, in the case of nucleus basalis, irregularly shaped. Thus, in this study, specificity may have been sacrificed for the sake of reproducibility by dissecting the regions containing projection nuclei along clear cut anatomical landmarks. Such a procedure ensured that the projection nuclei were included in the sample for biochemistry even if other structures were removed as well. The reproducibility of our dissections is reflected by the fact that the ChAT specific activity of any projection nuclei area from a given patient paralleled the ChAT specific activity in the corresponding projection field from that patient very closely. In fact, ChAT specific activity in septal versus hippocampal samples had an r2 value of 0.816 for all AD and control data, while ChAT specific activity in area 40 versus substantia innominata yielded an r2 value of 0.664 for all AD and control data or 0.981 if one control sample was omitted.

Because of our dissection method, however, the substantia innominata and septal nuclei samples taken may have included AChE within cholinergic or noncholinergic cells that were not a part of the nucleus basal, or the medial septal nucleus. The presence of this AChE could obscure the effects of AD on the AChE within the actual projection nuclei neurons. Substantia innominata data is further compromised by the small number of samples. Despite these limitations, our data for the two projection nuclei were remarkably consistent in showing no significant change in AChE molecular forms. We believe that our results strongly suggest that no large changes in AChE molecular form patterns occur in these presynaptic areas as a result of AD.

The results of this study in hippocampus and area 40 are consistent with the following view of AChE in areas of cholinergic termination. The AChE contained in cholinergic postsynaptic regions such as cortex and hippocampus derives from two sources. The majority of the activity appears to originate in axons carried from cholinergic nuclei, such as the nucleus basalis of Meynert and the medial septal nucleus but significant AChE activity is also contained in the cells within the postsynaptic region itself. Particularly in the case of the hippocampus, which contains few any intrinsic cholinergic neurons (7, 11, 17, 29), the postsynaptic AChE is contained within intrinsic noncholinergic neurons (7, 11, 35) or within noncholinergic axons traveling by pathways other than the fornix (7), and may play a role either in degrading molecules other than acetylcholine (8, 9) or in terminating cholinergic neurotransmission. The postsynaptic AChE can only be viewed when the pathways carrying axons from the presynaptic nuclei have either degenerated as in AD or been severed as in fornix-lesioned animals. If postsynaptic regions contain AChE with the same molecular form distribution as the presynaptic areas and if maintenance of this distribution is independent of cholinergic presynaptic input, then loss of the axons should decrease the total AChE activity but should not alter the distribution of its forms. This is obviously not the case in AD brains where changes in form distribution as well as loss of activity occur: studies of fornix-lesioned animals confirm the result (4, 38).

In conclusion, the major finding of this study is that AChE remaining in area 40 and hippocampus after the cholinergic denervation which occurs as part of the process of AD has a different molecular form composition than controls. In particular there is proportionally less of the membrane-bound G4 form which has critical importance for cholinergic neurotransmission. The intriguing question now is whether the AChE that is intrinsic to postsynaptic regions always contains a low proportion of membrane-bound G4, or whether the low G4 results from withdrawal of a modifying signal accompanying cholinergic innervation and serving to maintain normal levels of intrinsic postsynaptic membrane-bound G4.

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