Cholinergic Markers in Elderly Patients With Early Signs of Alzheimer Disease

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A central tenet of Alzheimer disease (AD), established 20 years ago and repeatedly replicated, is the loss of cortical cholinergic markers, specifically, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activity, in postmortem tissue from AD patients.1-3 This abnormality has been shown to correlate with neuropathological markers4 and with the severity of dementia.5,5 Therapies designed to reverse the cholinergic deficit, such as AChE inhibitors, are in large measure based on the importance of the cholinergic deficit to cognition and the symptoms of AD.6,8

Most postmortem studies assessing cholinergic markers in AD derive from patients with end-stage dementia. Those few studies in which brain biopsy specimens were obtained and cholinergic markers were assessed antemortem generally are restricted to patients with either a very early onset of dementia or relatively advanced dementia.3,9,10 Thus, whether these profound deficits in cholinergic markers found in end-stage patients apply to patients with much less severe disease is not clear. To study the earliest changes in AD, the Mount Sinai Alzheimer’s Disease Center, in collaboration with the Jewish Home and Hospital (both in New York, NY), has initiated a prospective study of its residents. Individuals who are relatively highly functioning are assessed at entry into the home and annually thereafter and followed up until their death, when attempts are made to have an autopsy performed. In this study, we evaluate the cholinergic markers of these patients with no cognitive impairment, questionable cognitive impairment, and early AD to address the relationship between early AD and cholinergic markers.

METHODS

Subjects

Eighty-one subjects were selected from 278 consecutive patients on whom autopsies were performed between 1986 and 1997 and who had been residents of the Jewish Home and Hospital in Manhattan and the Bronx, NY. Autopsies were performed after receiving institutional review board approval and consent for autopsy from each subject’s legal next of kin.

Clinical Dementia Rating Scale. First, subjects were assigned a Clinical Dementia Rating (CDR) Scale11,12 score using a multistep approach based

Context A central tenet of Alzheimer disease (AD) is the loss of cortical cholinergic function and cholinergic markers in postmortem brain specimens. Whether these profound deficits in cholinergic markers found in end-stage patients are also found in patients with much earlier disease is not known.

Objective To determine whether cholinergic deficits in AD precede, follow, or occur in synchrony with the earliest signs of cognitive deterioration.

Design, Setting, and Patients Postmortem study of nursing home residents with Clinical Dementia Rating (CDR) Scale scores of 0.0 to 2.0 and 4.0 to 5.0 who underwent autopsy between 1986 and 1997, comparing the activity of the cholinergic marker enzymes in the cortices of 66 elderly subjects with no (CDR score = 0.0; n = 18), questionable (CDR score = 0.5; n = 11), mild (CDR score = 1.0; n = 22), or moderate (CDR score = 2.0; n = 15) dementia vs subjects with severe dementia (CDR score = 4.0-5.0; n = 15).

Main Outcome Measures Activity of the cholinergic marker enzymes choline acetyltransferase and acetylcholinesterase in 9 neocortical brain regions.

Results The activity of choline acetyltransferase and acetylcholinesterase in 9 neocortical brain regions did not differ significantly in subjects with CDR scores of 0.0 to 2.0, but was significantly lower in subjects with severe dementia (CDR score = 4.0-5.0). Choline acetyltransferase levels were significantly correlated with severity of neuropathological lesions of AD, as measured by density of neuritic plaques and neurofibrillary tangles.

Conclusions Although neocortical cholinergic deficits are characteristic of severely demented AD patients, in this study, cholinergic deficits were not apparent in individuals with mild AD and were not present until relatively late in the course of the disease. These results suggest that patients with more severe disease should be a target for cholinergic treatment.

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on cognitive and functional status during the last 6 months of life. These steps involved (1) assignment of a CDR score based on a careful review of all information contained within each patient's chart; (2) a blinded review of the same records by a second reviewer experienced in neuropsychological assessment of living elderly patients and the assignment of a second independent CDR score; and (3) a telephone interview with at least 1 family member and/or caregiver for each subject and assignment of a third CDR score. All 3 CDR scores and all pertinent chart information were subsequently presented to a senior clinician (D.M.) and a consensus CDR score was derived.

**CDR Validation.** The reliability of the postmortem chart review procedure for CDR scoring was determined by direct observation and patient interview and by chart review alone for 35 subjects. An interclass correlation coefficient of 0.86 was obtained for the 2 independent assessments of CDR. A subset of the subjects (n = 22) had been neuropsychologically assessed during life and had participated in longitudinal studies of cognitive function with instruments such as the Mini-Mental State Examination and the Alzheimer's Disease Assessment Scale. When available, the neuropsychological assessment results were also considered in deriving the final consensus CDR score. The correlation between the consensus CDR score assigned and the Mini-Mental State Examination score for those 22 subjects who had been assessed antemortem was $r = -0.48$ ($P = .03$). If only those subjects who had received a Mini-Mental State Examination score within 1 year of death were considered (n = 14), then the correlation between the consensus CDR and the last Mini-Mental State Examination score rose to $r = -0.73$ ($P = .003$).

**Neuropathological Assessment**

The procedures used for the neuropathological assessments have been described previously. Initially, all subjects with non-AD neuropathology or AD neuropathology complicated with other neuropathological lesions of sufficient magnitude to contribute to cognitive dysfunction were excluded from consideration. These neuropathological lesions included but were not limited to Pick disease, diffuse Lewy body disease, Parkinson disease, stroke, multi-infarction dementia, and severe cerebrovascular disease. Subjects with mild cerebrovascular disease judged by our neuropathologists (D. P. Purohit and D. P. Perl) to be insufficient in severity to affect cognitive function were not excluded from further consideration.

The neuropathological assessment consisted of examining representative blocks from superior and midfrontal gyrus, orbital cortex, basal ganglia with basal forebrain, amygdala, hippocampus (rostral and caudal levels with adjacent parahippocampal and inferior temporal cortex), superior temporal gyrus, parietal cortex (angular gyrus), calcarine cortex, hypothalamus with mammillary bodies, thalamus, midbrain, pons, medulla, cerebellar vermis, and lateral cerebellar hemisphere. Sections from paraffin-embedded blocks were stained using hematoxylin-eosin, modified Bielschowsky, modified thioflavine S, anti-β-amyloid, and anti-τ. Every case was evaluated for the extent of neuropathological lesions using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropathological battery. The results of the neuropathological studies were used to exclude all cases that did not meet criteria as neuropathologically normal or subjects who had neuropathological lesions other than those associated with AD. Estimates of the densities of neurofibrillary tangles (NFTs) bodies from the CERAD battery and counts of neuritic plaque (NP) density in the regions of interest were used for correlational analysis with the activity of cholinergic marker enzymes. It should be noted that the neuropathological measures were obtained from examination of the right half of the brain whereas the neurochemical measures were obtained from cortical specimens derived from the left hemisphere.

**Final Case Selection**

After the completion of the neuropathological studies and assignment of consensus CDR scores, a final consensus conference was held (with the participation of K.L.D., R.C.M., D.M., D. P. Purohit, D. F. Perl, and V.H. and the antemortem and postmortem assessment team) to select cases for measurement of cholinergic markers and inclusion in this study. All patients with neuropathological lesions other than those of AD were excluded. Because the aim of this study was to identify the relationship of cortical cholinergic markers to early and mild dementia, only subjects with CDR scores of 0.0 to 2.0 were selected for inclusion. These were considered as 4 groups, having CDR scores of 0.0, 0.5, 1.0, and 2.0. An additional group of 15 subjects with CDR scores of 4.0 and 5.0 (referred to hereafter as CDR scores of 5.0) were included to represent severe dementia. These subjects were selected to match those with CDR scores of 0.0 to 2.0 as closely as possible with respect to age, sex, and time from death to autopsy. Subjects with a CDR score of 4.0 (n = 5) did not differ significantly from subjects whose CDR score was 5.0 (n = 10) with respect to any demographic, neuropathological, or neurochemical parameter measured.

**Measurement of Cholinergic Markers**

At the time of autopsy, the brain was halved midsagittally. The right half was fixed in 4% paraformaldehyde solution and was used for neuropathological evaluation as described herein. The left half was sectioned into 0.5- to 0.8-mm coronal slabs, flash frozen in liquid nitrogen-cooled isopentane, and stored at −80°C. The neocortical regions dissected for ChAT and AChE analysis corresponded to the middle frontal gyrus (Brodmann area 8); inferior frontal gyrus (Brodmann area 44); anterior cingulate gyrus (Brodmann area 32); superior, middle, and inferior temporal gyri (Brodmann areas 22, 21, and 20, respectively); the entorhinal cortex (Brodmann area 36/28); the inferior parietal...
lobule (Brodmann area 7); and the primary visual cortex (Brodmann area 17). The dissections of these regions were based on cortical maps similar to those published by Damasio and Damasio and were similar to the procedures described previously. One aliquot (approximately 100 mg) from each brain region of each subject was used for the ChAT and AChE activity assays. For 1 subject with a CDR score of 0.0, cortical tissue was available from only 3 Brodmann areas (20, 21, and 22). The procedures for the ChAT and AChE activity assays were identical to those already described and were modified from the procedures described by Fonnum and Johnson and Russell, respectively. The activity of ChAT and AChE was expressed as a function of protein concentration that was estimated by the method of Bradford.

**Data Analyses**

The 5 CDR categories were used as the independent variable for subsequent analyses. The dependent variables consisted of the activity of ChAT and AChE in each of the 9 cortical regions. Repeated-measures analyses of variance were used to analyze the activity of ChAT and AChE across cortical regions. Tukey tests were used for between-group comparisons. Pearson product-moment and Spearman rank-order correlations procedures were used to calculate the correlation of cholinergic marker enzyme activity in different cortical regions with CDR scores, NP densities, and ratings of NFT densities. For the correlational analyses, Bonferroni correction was applied where indicated by multiplying the P value calculated by the total number of analyses performed in that series.

**RESULTS**

The demographic characteristics of the final study cohort are presented in Table 1. Groups formed on the basis of CDR scores did not differ significantly with respect to age (F_{4,76} = 1.4; P > .25). Although there were significantly (P < .006) more women (n = 52) than men (n = 14) in the study cohort as a whole, the proportion of men and women in the different CDR groups did not differ significantly (χ² = 0.8; P = .82).

Subjects were grouped purely on the basis of the CDR scores described herein, without regard to neuropathological diagnosis of AD. However, the distribution of subjects on the basis of neuropathological diagnoses was roughly similar to their distribution along the cognitive dimension as determined by the CDR score. The number of subjects in each CDR category receiving neuropathological diagnoses of definite, probable, possible, and no AD is shown in Table 2.

**Activity of ChAT and AChE as a Function of Cognitive Status**

The activity of ChAT and AChE in the 9 cortical regions studied are shown in Figure 1 and Figure 2. Analysis of variance of ChAT activity revealed a significant effect of CDR groups (F_{4,74} = 17.57; P < .001), a significant effect of brain regions (F_{8,392} = 25.28; P < .001), and a significant CDR group-by-brain regions interaction (F_{32,592} = 2.49; P < .001). Analysis of the effect of CDR showed that the CDR 5.0 group differed significantly from all other groups (P < .001 for all) in all brain regions (P < .001 for all). No other differences among CDR groups were statistically significant (P > .10 for all). The relationship between CDR scores and AChE activity was nearly identical to the relationship between CDR scores and ChAT activity (data not shown). The significant differences among cortical regions were attributable to higher ChAT activity in Brodmann areas 21 and 36, higher AChE activity in Brodmann areas 20, 21, 22, and 36 relative to all other areas (P < .04 for all), and lower ChAT and AChE activity in the primary visual cortex relative to other cortical regions (P < .003 for all).

**Correlation of ChAT and AChE Activity With Cognitive Status**

The activity of ChAT correlated significantly with CDR scores (range, r = −.46 [Brodmann area 32] to r = −.05 [Brodmann area 22]; P < .001 for all after Bonferroni correction) when the entire cohort was considered. However, when subjects with CDR scores of 5.0 were excluded from the analysis, the ChAT activity did not correlate significantly with CDR scores in any of the cortical regions studied (range, r = −.07 [Brodmann area 8] to r = −.27 [Brodmann area 20]; P > .05 for all after Bonferroni correction). Figure 3 shows the correlation of ChAT activity in the superior...
temporal gyrus (Brodmann area 20) with CDR scores for the entire study cohort. Identical relationships were observed when correlations between AChE activity and CDR scores were considered.

Correlations of ChAT and AChE Activity With Severity of Neuropathological Lesions

As part of the neuropathological assessment of the subjects, the density of NPs15 and NFTs16 was determined in several cortical regions, including the middle frontal gyrus (Brodmann area 8), the superior temporal gyrus (Brodmann area 22), the inferior parietal lobule (Brodmann area 7), and the primary visual cortex (Brodmann area 17). When the entire cohort was used, the density of plaques in each cortical region correlated significantly with the activity of ChAT and AChE in that region (range, \( r = -0.27 \) to \( r = -0.47 \) by Pearson product-moment correlation; \( P < .02 \) for all). FIGURE 4 provides an example of 1 of these correlations, in which the NP densities in the superior temporal gyrus are plotted against the activity of ChAT in Brodmann area 22. Similar correlations were observed between the activity of ChAT and AChE and the NFT densities in each cortical region (range, \( r = -0.26 \) to \( r = -0.63 \) by Spearman rank-order correlation; \( P < .02 \) for all). FIGURE 5 shows the correlation of NFT densities in the superior temporal cortex with the activity of ChAT in Brodmann area 22. For NPs, the significant correlations between NPs and the activity of ChAT and AChE were due in large part to the inclusion of subjects with CDR scores of 5.0. When the CDR 5.0 group was excluded from the analyses, only the correlation between ChAT activity and NP density in the middle frontal gyrus was significant (\( r = -0.34 \); \( P = .03 \) after Bonferroni correction). When a similar analysis was performed for NFT densities (eliminating the CDR 5.0 cohort), only the correlation between ChAT activity and NFT density in the superior temporal gyrus remained statistically significant (\( r = -0.40 \), \( P = .03 \) after Bonferroni correction).

Activity of ChAT and AChE in Subjects With Neuropathological Diagnosis of AD

The activity of ChAT and AChE was significantly reduced if subjects were grouped purely on the basis of neuropathological diagnosis. Analysis of variance for the activity of ChAT in the 9 cortical regions of subjects neuropathologically diagnosed with AD showed significant reductions (\( P < .001 \) for all) in the CDR 5.0 group only. BA indicates Brodmann area.
logically characterized as not having AD vs definitely having AD revealed a significant effect of diagnosis ($F_{1,90} = 11.35; P = .002$). Choline acetyltransferase activity was significantly reduced in Brodmann areas 8, 20, 21, 22, and 36 in the group neuropathologically diagnosed as definitely having AD relative to neuropathologically healthy controls ($P<.03$ for all). Identical results were obtained for AChE activity. However, when the regional activity of ChAT and AChE in the 13 neuropathologically healthy elderly subjects with CDR scores of 0.0 were compared with the regional activity of ChAT and AChE in subjects with CDR scores of 0.5 and 1.0 and neuropathological diagnoses of definite and probable AD, no significant group differences were found ($F_{1,23} = 0.5; P = .48$ for ChAT and $F_{1,23} = 0.52; P = .48$ for AChE). The activity of ChAT and AChE correlated significantly with each other in each cortical region. The overall correlation between ChAT and AChE activity was $r = 0.67$. Regional analysis showed that the correlation between ChAT and ChE activity was highest in Brodmann area 21 of the temporal cortex ($r = 0.81; P < .001$) and lowest in Brodmann area 36 of the temporal cortex ($r = 0.53; P < .001$).

**COMMENT**

There is little evidence in the current sample that deficits in ChAT or AChE are early markers of AD. Only the CDR 5.0 group differed from the other CDR groups in ChAT or AChE activity. Subjects having a CDR score of 1.0, 50% of whom met neuropathological criteria for definite AD (Table 2), did not have cholinergic marker activity that was significantly lower than the control group. Even if only those patients with a CDR score of 1.0 who meet neuropathological criteria for definite AD are considered, they still did not have a significant reduction in cholinergic markers ($P = .81$). Of even greater surprise is the failure of the group with a CDR score of 2.0 (patients with moderate AD dementia) to differ significantly from the control group ($F_{1,29} = 3.7; P = .06$), although there was a clear trend toward lowered cholinergic markers at this level of dementia.

It is noteworthy that pathological markers did not robustly distinguish those patients who have higher ChAT or AChE activity in the groups with CDR scores of 0.0, 1.0, and 2.0 from those subjects who have lower ChAT activity. When the subjects in the groups with CDR scores of 0.0 to 2.0 were divided into those with mean cortical NP densities of less than 10/mm² vs those with at least 10/mm² (Khachaturian criteria for AD in subjects aged 66-75 years), the regional activity of ChAT was slightly lower in the high-plaque group relative to the low-plaque group (mean ChAT activity, 12.7 vs 10.7 nmol of acetylcholine per milligram of protein per hour, respectively), but the differences did not approach statistical significance ($F_{1,27} = 0.35; P = .56$). These conclusions are supported further by the lack of significant correlation of the NP and NFT densities with the activity of ChAT and AChE in subjects with mild and moderate dementia. Hence, the presence of the neuropathological characteristics of AD is not a strong contributor to cholinergic function in subjects with mild dementia. On the other hand, even mild or questionable dementia was associated with significant increase in cortical NP and NFT densities. This contrasts rather substantially with the case for ChAT and AChE activity in which statistically significant deficits are apparently not seen, in this sample, until a CDR score of greater than 2.0 is reached. These data suggest that plaques are an earlier marker of AD than cholinergic markers. In further support of this conclusion, although ChAT or AChE activity and NP density correlated significantly with each other (Figure 4), these correlations did not reach statistical significance without the inclusion of the CDR 5.0 group. Thus, among the distribution of controls and patients with questionable, definite but early, and moderate AD cognitive deficits, no relationship with cholinergic markers and NP density was found in any region studied.

It is clear from these data that it is unlikely that a cholinergic marker would be an early indicator of AD, at least in patients of this older age group. It is even less likely that a cholinergic deficit could be identified prior to patients becoming symptomatic. These observations have important implications for the use of cholinomimetics and, particularly, AChE inhibitors, in the treatment of AD. Most patients included in study groups that test the efficacy of cholinesterase inhibitors would fall into the CDR score range of 1.0 or 2.0 in the current study and would have a mean age in the early 70s. It is well recognized that dementia with onset as
early as the fifth and sixth decades of life differs from the more common kind of AD found in the eighth or ninth decades of life on a number of important dimensions, including heritability and course.24,25 As a group, these younger patients have a modest response to cholinesterase inhibition.8 Because they are considered considerably younger than the current series of patients, they might have more extensive cholinergic lesions.3 Alternatively, such patients are reminiscent of healthy young people who previously have been shown to respond to physostigmine with an increase in their ability to learn new information.20 It is possible that the efficacy of cholinomimetics in patients with AD of only mild to moderate degree results from boosting cholinergic activity to levels higher than the patients’ premorbid baseline rather than from reversing a cholinergic deficit. On the other hand, these data certainly suggest that more robust effects with cholinomimetics might be found in patients with more severe disease, i.e., patients with CDR scores of at least 2.0.

Indeed, greater response to cholinomimetics has been reported in patients with more severe illness.72 However, such patients commonly have Mini-Mental State Examination scores of less than 10 and have not been extensively studied for the efficacy of cholinomimetics. Given the presence of NP and NFT pathological findings in mild dementia and the absence of a cholinergic deficit, the question remains as to the neurochemical nature of the cognitive deficit. As noradrenergic and serotonergic deficiencies are more commonly seen in younger patients and the current study population constituted a group with a mean age beyond that at which noradrenergic and serotonergic abnormalities are found, it is unlikely that these neurotransmitters could have been the substrate for the cognitive deficits in the population with mild dementia. However, it is possible that corticocorticon-releasing factor, somatostatin, or a subgroup of glutamatergic neurons might be affected in these early AD groups.3 In addition, although the neocortical activity of ChAT and AChE did not decrease in subjects with CDR scores of 0.5, 1.0, and 2.0, some degeneration of the forebrain cholinergic system still could have occurred. It is possible that a subset of forebrain cholinergic neurons degenerated but compensatory changes in the remaining neurons normalized neocortical ChAT and AChE activity.25-30 Detailed stereological studies of the forebrain cholinergic neurons will have to be performed to address this possibility conclusively.

Although neocortical cholinergic deficits are characteristic of severely demented AD patients, in this study, cholinergic deficits were not detected in individuals with mild AD and were not present until relatively late in the course of the disease. These results suggest that patients with more severe disease should be a target for cholinergic treatment.

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### References