Preservation of Brain Nerve Growth Factor in Mild Cognitive Impairment and Alzheimer Disease

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Background: The status of nerve growth factor (NGF) levels during the prodromal phase of Alzheimer disease (AD), characterized by mild cognitive impairment (MCI), remains unknown.

Objective: To investigate whether cortical and/or hippocampal NGF levels are altered in subjects with MCI or different levels of AD severity.

Design and Main Outcome Measures: An NGF enzyme-linked immunosorbent assay determined protein levels in the hippocampus and 5 cortical areas in people clinically diagnosed as having no cognitive impairment, MCI, mild AD, or severe AD.

Results: We found no changes in cortical or hippocampal NGF levels across groups; in MCI, levels did not correlate with an increase in choline acetyltransferase activity in these regions.

Conclusion: Brain NGF levels appear sufficient to support the cholinergic plasticity changes seen in MCI and remain stable throughout the disease course.

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It has been hypothesized that cholinergic basal forebrain (CBF) cortical and hippocampal projection neurons degenerate in Alzheimer disease (AD) because of the loss of neurotrophic support from their target sites, which produce nerve growth factor (NGF).1 This protein has well-known survival effects on CBF neurons.2,3 Studies have reported unchanged,4-7 decreased,8 or increased8-13 NGF levels in the cortex and hippocampus in patients with end-stage AD. Despite these inconsistencies and the initiation of a clinical trial to test the effect of NGF gene therapy as a treatment for mild AD,14 there are scant data concerning alterations in NGF levels in the cortex and hippocampus throughout the course of AD. Interestingly, choline acetyltransferase (ChAT) activity is increased in the frontal cortex and hippocampus in people with mild cognitive impairment (MCI).15,16 To determine whether NGF levels exhibit a similar plasticity response, we evaluated these levels in the hippocampus and 5 cortical areas in people clinically diagnosed as having no cognitive impairment (NCI), MCI, mild AD, or severe AD.

METHODS

We evaluated 54 individuals (Table 1) who participated in a longitudinal study of aging and AD among the Catholic clergy, the Religious Orders Study (ROS).15,17-20 Subjects were categorized as having NCI, MCI, or mild AD. Twelve subjects with severe AD were selected from the University of Pittsburgh Alzheimer’s Disease Research Center (Pittsburgh, Pa). At their last evaluation (performed within 12 months of death), they had a mean Mini-Mental State Examination score of 8.3 (significantly lower than all 3 ROS groups; P<.001). These subjects were similar to the 3 ROS groups in age at death, sex, level of education, APOE e4 status, and postmortem delay (Table 1). The human investigations committees of Rush Presbyterian–St Luke’s Medical Center (Chicago, Ill) and the University of Pittsburgh approved this study.

CLINICAL EVALUATION

Details of the clinical evaluation in the ROS and University of Pittsburgh cases have been published elsewhere.15,17,21 Our MCI population was defined as subjects rated as impaired on neuropsychological testing but not having dementia.17,20 The University of Pittsburgh patients were diagnosed as having AD following a stan-
Tissue collection and processing were virtually identical for ROS and University of Pittsburgh subjects.\(^15,17-19,21\) Cases were included from these studies if brain abnormalities were substantial. Tissue was snap-frozen in liquid nitrogen and frozen at \(-80^\circ\text{C}\) until analysis. All cases were assigned a neuropathologic diagnosis based on Braak and Braak staging scores\(^24\) and National Institute on Aging (NIA)–Reagan criteria\(^25\) and genetic diagnosis based on Braak and Braak staging scores\(^24\) and National Institute on Aging (NIA)–Reagan criteria\(^25\) (now the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision).\(^26\)

**Tissue Preparation and Neuropathologic Designation**

Brain tissue collection and processing were virtually identical for ROS and University of Pittsburgh subjects.\(^15,17-19,21\) Cases were excluded from these studies if brain abnormalities were substantial. Tissue was snap frozen in liquid nitrogen and frozen at \(-80^\circ\text{C}\) until analysis. All cases were assigned a neuropathologic diagnosis based on Braak and Braak staging scores\(^24\) and National Institute on Aging (NIA)–Reagan criteria\(^25\) and genotyped for apolipoprotein E (APOE) using restriction isotyping\(^26\) and National Institute on Aging (NIA)–Reagan criteria\(^25\) and genotyped for apolipoprotein E (APOE) using restriction isotyping of genomic DNA isolated from plasma, performed by polymerase chain reaction amplification.\(^18,21,26\)

Designations of normal and apolipoprotein E (APOE) were typed for apolipoprotein E (APOE) and University of Pittsburgh subjects.\(^15,17-19,21\) Cases were included from these studies if brain abnormalities were substantial. Tissue was snap-frozen in liquid nitrogen and frozen at \(-80^\circ\text{C}\) until analysis. All cases were assigned a neuropathologic diagnosis based on Braak and Braak staging scores\(^24\) and National Institute on Aging (NIA)–Reagan criteria\(^25\) and genetic diagnosis based on Braak and Braak staging scores\(^24\) and National Institute on Aging (NIA)–Reagan criteria\(^25\) (now the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision).\(^26\)

**NGF Assay**

Homogenate samples were prepared using brain tissue sonication in phosphate buffer saline (pH, 7.2) containing protease inhibitors. An aliquot was then removed for total protein determination (BCA Protein Assay Kit; Pierce Biotechnology, Inc, Rockford, Ill). The remaining tissue lysate was centrifuged at 17,000g for 60 minutes, and NGF was measured in the resulting supernatant using a sandwich enzyme immunoassay. All tissue samples were run in triplicate. The capture antibody was monoclonal antibody anti-β (2.5 Svedberg flotation units [SI]; 7 SI) NGF, whereas the detection antibody was monoclonal antibody anti-β (2.5 SI; 7 SI) NGF-β-Gal, a β-galactosidase–conjugated form of the capture antibody (both from Roche Diagnostics, Mannheim, Germany). Detailed assay description and the sensitivity and specificity of human NGF antibodies have been published previously.\(^8,28,29\) The amount of NGF present in tissue samples was determined by monitoring the color change of a substrate solution (chlorophenol red galactopyranoside; Sigma Chemical, St Louis, Mo) at 570 nm using a plate reader (Dynatech, Vienna, Va) and comparing the results with human NGF standards (Roche Diagnostics) run in parallel with the same plate.

To control for any nonspecific binding of NGF in the tissue lysates, tissue samples were spiked with a known amount of NGF standard, and the percentage of NGF recovered corrected the reading value. Samples from all 4 diagnostic groups were run in parallel. To ensure reproducibility of the assay, randomly chosen samples were run using multiple plates, yielding comparable values for the same sample. The NGF measurements were expressed as picograms of NGF per milligrams of protein.

**Statistical Analyses**

Demographic and clinical characteristics of diagnostic groups were compared using analysis of variance for continuous variables and the Fisher exact test for categorical variables; pairwise comparisons were performed using the Tukey test. To assess NGF levels across diagnostic groups, we used the nonparametric Kruskal-Wallis test. Distribution of neuropathologic lesions according to NIA-Reagan criteria and Braak and Braak staging was compared using the Fisher exact test. Correlations were performed using the Spearman rank correlation. Statistical significance was set at \(P<.05\) (2-tailed test).
RESULTS

DEMOGRAPHICS

Individuals with NCI, MCI, or mild AD were similar in age, education level, sex representation, and postmortem interval (Table 1). Pairwise comparisons showed that the difference in Mini-Mental State Examination score among the 3 ROS groups \((P<.001)\) was primarily due to lower scores in the AD group \((P=.04)\). Of the mild AD cases, 42% contained at least 1 APOE-ε4 allele compared with 28% for the MCI group and 20% for the NCI group (Table 1); the differences were not statistically significant. Of the patients with end-stage AD, 90% had at least 1 ε4 allele.

BRAIN NGF LEVELS

There were no differences in NGF levels across clinical groups for any of the cortical regions examined (Figure 1 and Table 2). The hippocampus of the subjects with mild AD contained the highest NGF levels (Table 2); levels were not statistically different among the 3 clinically diagnostic groups in the ROS. Mean ± SD NGF levels were 13.4 ± 5.5 for the middle temporal cortex and 25.9 ± 13.9 for the hippocampus in patients with end-stage AD. No significant differences in NGF levels were found when comparing severe AD cases with any of the ROS groups examined.

NGF LEVELS, ChAT ACTIVITY, AND NUCLEUS BASALIS OF MEYNERT NEURON COUNTS

We found no correlations between cortical or hippocampal NGF levels and ChAT activity (Figure 2). We also found decreases in nucleus basalis of Meynert neurons containing ChAT, tyrosine receptor kinase A, or low-affinity neurotrophin receptor, as derived from previous studies.\(^{15,17,18,21}\) We have not performed cell counts of cholinergic septal diagonal band–hippocampal projection neurons, so we were unable to determine the correlations with hippocampal NGF levels.

NGF LEVELS VS COGNITIVE SCORES

For each brain region examined, we evaluated the associations between NGF levels and scores on cognitive tests referable to these regions. Scores for these analyses were obtained from the last annual evaluation, performed within 1 year of death. Hippocampal (Spearman rank correlation; \(r=-0.31; P=.04\)) and middle temporal cortex (Spearman rank correlation; \(r=-0.35; P=.01\)) NGF levels were negatively correlated with Word List Recogni-
Test1 scores; superior frontal cortex levels were positively correlated with these scores (Spearman rank correlation; \( r = 0.29; P = .04 \)). The NGF levels and cognitive functions in other brain regions had no statistically significant correlations (data not shown).

**NGF LEVELS VS REGIONAL QUANTITATIVE PATHOLOGIC FEATURES**

For 3 cortical regions (superior frontal, superior temporal, and inferior parietal), neuropathologic plaque counts were determined; no association between mean NGF levels was found. Across the clinical spectrum of severity, there were no differences based on Braak and Braak staging or NIA-Reagan criteria (Figure 3).

**COMMENT**

The NGF levels in the cortex and hippocampus of people with MCI, mild AD, or severe AD are stable, consistent with most other studies showing stability of NGF in end-stage AD.\(^3\) In this study, clinical information and diagnosis were based on a standard, uniform clinical evaluation prior to death.\(^2\) Therefore, this approach was not
confounded by selection bias resulting from case categorization and selection of subjects using neuropathologic criteria. In contrast, the earlier study^2^ clinically defined its subjects by a retrospective evaluation of case notes and postmortem interviews of relatives and medical staff as well as the display of β-amyloid 4 cortical plaques, none of which may provide an accurate representation of a patient’s clinical status. These methodological differences make it difficult to compare observations between these studies.

Prior studies indicating an increase in NGF messenger RNA and protein were performed in cases of end-stage AD.6–13 Scott et al.14 reported a wide range of NGF-like activity in a population of patients who had early-to late-onset AD. In their cohort some of the highest and lowest levels of NGF were seen in end-stage AD, suggesting that within a given disease cohort, NGF levels can be differentially affected by age at disease onset. Despite differences in age at onset, we and others15 found no correlation between ChAT activity and NGF levels, nor between the reduction in the number of neurons containing ChAT,11 tyrosine receptor kinase A,16 or low-affinity neurotrophin receptor and the NGF levels in MCI and mild AD. Moreover, the lack of correlation between any APOE ε4 genotype and NGF levels across disease stages is interesting because APOE ε3 and ε4 alleles are associated with a greater reduction in cholinergic markers in end-stage AD.30 The similarity in the degree of pathologic characteristics between the MCI and AD groups indicates that global AD-like pathologic features generally do not induce changes in NGF regulation within the septohippocampal or basocortical projection systems early in the disease state. These findings are consistent with other investigations demonstrating that the underlying pathologic characteristics of MCI are similar to those seen in AD.15,16 This suggests that AD-like abnormalities do not produce alterations in the cholinotrophic projection systems during the prodromal stage of the disease process. Taken together, these observations do not support the hypothesis that alterations in the central cholinergic cortical projection system affect NGF levels during the disease progression.

We previously demonstrated an up-regulation of ChAT activity in the hippocampus and superior frontal cortex in MCI,15,16 but there was no concomitant increase in NGF concentration in these or other regions examined in the present study. Elevation of hippocampal ChAT activity in MCI may be due to a cholinergic plasticity response related to the degeneration of entorhinal stellate neurons early in the disease,19,31 which induces sprouting of septal cholinotrophic projections into the denervated hippocampus.32,33

Finally, because NGF levels in CBF cortical target sites are unchanged across all stages of AD, it is unlikely that a simple defect in NGF production underlies the reduction of this protein seen in CBF neurons in end-stage AD.15 The stability of brain NGF levels appears sufficient to support the cholinergic plasticity response seen in MCI.15,16

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