Neuropeptide Abnormalities in Patients With Early Alzheimer Disease

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Background: Deficits in somatostatin-like immunoreactivity (SLI) and corticotropin-releasing factor immunoreactivity (CRF-IR) are well recognized as prominent neurochemical deficits in Alzheimer disease (AD). The question of whether these profound neuropeptidergic deficits found in patients with end-stage disease extend into those with much earlier disease is relatively unanswered. To determine the relation between level of SLI and CRF-IR in different cerebrocortical regions to the earliest signs of cognitive deterioration in AD.

Methods: We examined SLI and CRF-IR levels in 9 neocortical brain regions of 66 elderly patients in a postmortem study of nursing home residents who had either no significant neuropathologic lesions or lesions associated only with AD. Patients were assessed by the Clinical Dementia Rating scale (CDR) to have no dementia or questionable, mild, or moderate dementia, and were compared with 15 patients with severe dementia.

Results: Both CRF-IR and SLI were significantly reduced in the cortices of patients with the most severe dementia, but only the levels of CRF-IR were reduced in those with mild (CDR = 1.0) and moderate dementia (CDR = 2.0). Levels of CRF-IR and SLI correlated significantly with CDR, but this correlation was more robust for CRF-IR and persisted even when severely cognitively impaired patients were eliminated from analysis.

Conclusions: Although SLI and CRF-IR levels are significantly reduced in patients with severe dementia, only CRF-IR is reduced significantly in the cortices of those with mild dementia. Thus, CRF-IR can serve as a potential neurochemical marker of early dementia and possibly early AD.

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SUBJECTS AND METHODS

SUBJECTS

Brain specimens were obtained from 81 patients who had been residents of the Jewish Home and Hospital in Manhattan and the Bronx, NY. The specimens were selected from a larger group of 278 consecutive autopsies performed between 1986 and 1997. Autopsies were performed after receiving consent from each subject's legal next-of-kin. The methods used for subject selection and cognitive and neuropathologic assessment have been described previously.

NEUROPATHOLOGIC ASSESSMENT

Every case was evaluated for the extent of neuropathologic lesions using the Consortium to Establish a Registry for Alzheimer’s Disease neuropathologic battery. The densities of NPs and NFTs were determined in the middle frontal gyrus (Brodmann area 8), superior temporal gyrus (Brodmann area 22), inferior parietal lobe (Brodmann area 7), and primary visual cortex (Brodmann area 17). All patients, with non-AD neuropathologic or AD neuropathologic lesions, complicated with other neuropathologic lesions of sufficient magnitude to contribute to cognitive dysfunction (eg, Pick disease, diffuse Lewy body disease, Parkinson disease, stroke, multi-infarct dementia, and severe cerebrovascular disease), were excluded from consideration.

RESULTS

LEVELS OF CRF-IR IN DIFFERENT CDR GROUPS

Analysis of variance of CRF-IR revealed a significant effect of CDR groups ($F_{1,74} = 14.09, P < .001$), a significant effect of brain regions ($F_{8,992} = 23.89, P < .001$), and a significant CDR group by brain region interaction ($F_{32,992} = 1.96, P < .002; Figure 1$). Newman-Keuls analysis of the effect of CDR showed that the CDR = 0.0 group differed significantly from the CDR 1.0, 2.0, and 5.0 groups ($P < .02$ for all) across all brain regions. There were no significant differences in CRF-IR between the CDR 1.0 and CDR 2.0 groups, but both differed significantly from the CDR 4.0 to 5.0 group. The CDR 0.5 group did not differ significantly from the CDR 0.0 group ($P > .3$).

Significant differences between cortical regions were attributable to higher levels of CRF-IR in the superior temporal gyrus (Brodmann area 22) relative to other neocortical regions. Levels of CRF in the inferior temporal gyrus (Brodmann area 20) and occipital cortex (Brodmann area 17) were significantly lower than levels of CRF-IR in all other cortical regions examined ($P < .01$).

Results of neuropathologic studies of NP and NFT density were used to determine the correlation of NP and NFT density with CRF-IR in each of the neuropathologically assessed brain regions. When the entire cohort was used, the density of plaques in each cortical region correlated significantly with CRF-IR in that region (Pearson product-moment correlation, $r = -0.26$ to $-0.33, P < .02$ for all; Figure 2). Similar correlations, albeit slightly higher, were observed between CRF-IR level and the density of NFTs in each cortical region (Spearman rank correlations, $r = -0.23$ to $-0.45, P < .04$ for all; Figure 3). Significant correlations of NPs and NFTs with CRF-IR were due in large part to the inclusion of the patients with CDR scores of 5.0. When the CDR = 5.0 group

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correlation coefficient of 0.86 was obtained for the 2 independent assessments of CDR. Twenty-two patients had been neuropsychologically assessed and had participated in longitudinal studies of cognitive function with instruments such as the Mini-Mental State Examination (MMSE) and the Alzheimer’s Disease Assessment Scale. The correlation between the consensus CDR and MMSE scores was \( r = -0.48 \) (\( P < .02 \)). If only those subjects who had received an MMSE score within 1 year of death were considered (\( n = 14 \)), then the correlation between the consensus CDR and the last MMSE increased to \( r = -0.73 \) (\( P < .003 \)). Subjects were grouped purely on the basis of CDR score, without regard to neuropsychologic diagnosis of AD. The distribution of subjects on the basis of neuropsychologic diagnoses was roughly similar to their distribution along the cognitive dimension (Table 1). Groups 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 to women did not differ significantly (\( \chi^2 = 0.8, P > .8 \)) and there were no sex-related differences between any neurochemical or neuropathologic measure studied (\( P > .20 \) for all).

**TISSUE HANDLING AND NEUROCHEMICAL PROCEDURES**

The procedures for tissue handling and preparation have been described previously. Neuropathologic examination was performed on the paraformaldehyde-fixed right half of the brain. Neocortical regions dissected for somatostatin and CRF analysis were derived from the flash-frozen left half of the brain, and corresponded to the middle frontal gyrus (Brodmann area 8); inferior frontal gyrus (Brodmann area 44); anterior cingulate gyrus at the level of the genu of the corpus callosum (Brodmann area 24/32, referred to as Brodmann area 32); superior, middle, and inferior temporal gyri (Brodmann areas 22, 21, and 20, respectively); entorhinal cortex (Brodmann area 36/28); inferior parietal lobule (Brodmann area 7); and primary visual cortex (Brodmann area 17). For one subject in the CDR = 0.0 group, cortical tissue was available from only 3 Brodmann areas (20, 21, and 22); for another, specimens were available for all except Brodmann areas 8, 32, and 44. The procedures for radioimmunoassay of SLI and CRF-IR have been described previously. The somatostatin antisera were directed toward residues 6 to 10 of the somatostatin (1-14) molecule and recognize somatostatin (1-14) and somatostatin (1-28) on an equimolar basis. Tissue concentrations of CRF-IR were determined using radioimmunoassay materials purchased from Peninsula Laboratories (Belmont, Calif; standard 8561; tracer Y-8562; antisera RAS-8561N). Protein was estimated by the method of Bradford.

**DATA ANALYSES**

The 5 CDR categories (0.0, 0.5, 1.0, 2.0, 4.0, and 5.0) were used as the independent variables, whereas the dependent variables consisted of the levels of SLI and CRF-IR in each of the 9 cortical regions. Repeated-measures analyses of variance were used to analyze the levels of CRF-IR and SLI across cortical regions. Newman-Keuls tests were used for between-group comparisons. Multiple regression analysis was used to evaluate the association of CDR score with CRF-IR and SLI in all 9 cortical regions. The Scheffé procedure for multiple comparisons was used to test these associations in single or subsets of cortical regions. Pearson product moment and Spearman rank order correlation procedures were used to determine the correlation between SLI, CRF-IR, NP densities, and ratings of NFT densities in selected cortical regions. For examination of correlations of CRF-IR and SLI levels in individual regions with CDR or the neuropathologic variables, a Bonferroni correction to a significance level of .05/9 = .006 was used. Statistical analyses were performed using Statistica for Windows, version 5.0 (StatSoft Inc, Tulsa, Okla) and SPSS for Windows, versions 7.5 and 9.0 (SPSS Inc, Chicago, Ill).

was excluded from analyses, no correlation between CRF-IR and NP and NFT density remained significant. Similar results were obtained for SLI in the 5 CDR groups; however, decreases in SLI were less sensitive to cognitive deficits than the CRF-IR decreases shown above.

**LEVELS OF SLI IN DIFFERENT CDR GROUPS**

There was a significant decrease in SLI as a function of CDR groups (\( F_{4,76} = 6.78, P < .001 \)), and significant SLI concentration differences in the different cortical regions (\( F_{8,592} = 93.5, P < .001 \)). The interaction term for CDR and cortical regions was not significant, however (\( F_{32,592} = 1.08, P > .35 \)). Newman-Keuls analysis of the effects of CDR grouping on SLI across the cortical regions showed that SLI concentrations were significantly (\( P < .02 \) for all) lower in the CDR 4.0 to 5.0 group relative to each of the other groups. The CDR 0.0, 0.5, 1.0, and 2.0 groups did not differ significantly from each other (\( P > .12 \) for all). As evident from Figure 4, the concentrations of SLI in the different cortical regions differed greatly and significantly from each other. The highest concentrations of SLI were detected in the temporal neocortex, whereas the lowest concentration was found in the occipital cortex (Brodmann area 17). Newman-Keuls analyses showed that the levels of SLI differed significantly (\( P < .03 \) for all) between virtually every pair of cortical regions examined.

Level of SLI significantly correlated with NP densities in the superior frontal gyrus (Brodmann area 8, \( r = -0.28, P < .05 \)), superior temporal gyrus (Brodmann area 22, \( r = 0.29, P < .04 \)), and occipital cortex (Brodmann area 17, \( r = -0.35, P < .01 \)). Similarly, SLI levels significantly and negatively correlated with the density of NFTs in the superior frontal gyrus (\( r = -0.30, P < .04 \)), superior temporal gyrus (\( r = -0.32, P < .02 \)), and inferior parietal lobule (\( r = -0.36, P < .004 \)). Levels of SLI and NP and NFT densities did not correlate significantly after Bonferroni correction in the remaining regions common to both measures. The correlation of SLI levels and neuropathologic markers was almost entirely due to the decreases in SLI levels observed in the CDR 4.0 to 5.0 group. When this group was excluded from analyses, no correlation reported above approached statistical significance.
CORRELATION OF CRF-IR AND SLI LEVELS WITH CDR SCORES

Multiple regression analysis with CDR as the dependent measure, and levels of CRF-IR and SLI in the 9 cortical regions as the sets of independent variables, showed that the levels of each peptide correlated significantly with CDR (CRF-IR $R^2 = 0.49$, $F_{9,69} = 7.3$, $P = .001$; SLI $R^2 = 0.37$, $F_{9,69} = 4.6$, $P < .001$). Simple correlation analyses showed that the levels of CRF-IR in most regions correlated significantly ($P < .05$ for all, after Bonferroni correction) and negatively with CDR scores (Table 2 and Figure 5). Stepwise regression showed that the level of CRF-IR in the middle temporal gyrus (Brodmann area 21) was the best predictor of CDR ($R^2 = 0.35$, $F_{9,69} = 5.2$, $P < .001$), with the level of CRF-IR in the inferior frontal gyrus (Brodmann area 44) making an additional significant contribution ($R^2$ for CRF-IR in Brodmann area 21 plus CRF levels in Brodmann area 44 = 0.45; $F_{9,69} = 6.8$, $P < .001$). For SLI, the region of strongest predictive power for CDR was the occipital cortex (Brodmann area 17; $R^2 = 0.26$, $F_{9,69} = 3.2$, $P = .003$). Significant correlations between CDR and the levels of SLI and CRF-IR in other regions were present (Table 2) but did not add significantly to the predictive power of CRF-IR and SLI to CDR. Identical analyses limited to the CDR 0.0 and 2.0 groups yielded a significant relationship between CDR and CRF-IR ($R^2 = 0.34$; $F_{9,54} = 3.0$, $P = .005$), but not between CDR and SLI ($R^2 = 0.15$; $F_{9,54} = 1.1$, $P = .41$). Stepwise regression analysis for CRF-IR showed the entorhinal cortex (Brodmann area 36/38) to be the principal region contributing to the regression results, but this regression did not reach statistical significance after correction for all possible contrasts ($R^2 = 0.19$; $F_{9,54} = 1.77$, $P = .1$). Combined regression analysis of CRF-IR and SLI levels as predictors of CDR ($R^2 = 0.57$) for the entire cohort showed that,

Table 1. Demographic and Neuropathologic Characteristics of the Study Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Clinical Dementia Rating Scale Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Age, mean ± SD, y (range)</td>
<td>83.8 ± 9.9 (64-99)</td>
</tr>
<tr>
<td>Male, mean age, y/N</td>
<td>82.3/3</td>
</tr>
<tr>
<td>Female, mean age, y/N</td>
<td>84.1/15</td>
</tr>
<tr>
<td>PMI, mean ± SD, h</td>
<td>8.29 ± 5.96</td>
</tr>
<tr>
<td>Healthy</td>
<td>13</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>0</td>
</tr>
<tr>
<td>Definite</td>
<td>1</td>
</tr>
<tr>
<td>Probable</td>
<td>4</td>
</tr>
<tr>
<td>Possible</td>
<td>15</td>
</tr>
<tr>
<td>Total No.</td>
<td>18</td>
</tr>
</tbody>
</table>

* PMI indicates postmortem interval.

Figure 1. Corticotropin-releasing factor immunoreactivity (CRF-IR) (expressed as picograms per milligrams of protein) in 9 cortical regions as a function of cognitive status (Clinical Dementia Rating scale [CDR]) during the 6 months before death. Data are expressed as mean ± SEM. BM indicates Brodmann area. See Table 1 for sample sizes and demographic characteristics.

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when SLI was entered first, the addition of CRF-IR significantly increased the correlation from $R^2 = 0.37$ to $R^2 = 0.57$ ($F_{9,60} = 3.0, P = .006$), but when CRF-IR was entered into the regression equation first, the addition of SLI did not increase the correlation significantly from $R^2 = 0.49$ to $R^2 = 0.57$ (CRF-IR followed by SLI, $F_{9,60} = 1.2, P = .30$).

COMMENT

This study was not designed to reaffirm what has been well established, namely, that CRF-IR and SLI are diminished in patients with AD. Rather, it was designed to determine how early in the progression of the disease these markers might be present in postmortem tissue of patients with AD. Therefore, patients with a definite but early stage of dementia (CDR = 1.0) are in many ways the most informative. Patients with CDR 0.5 are potentially interesting, but a substantial percentage of them can be expected to not have AD, even in a very early form. Indeed, 36% of this group did not have pathologic changes denoting even possible AD (Table 1). Patients with CDR = 2.0 are of interest only to the extent that when a peptidergic abnormality such as SLI is not present in them, it becomes possible to conclude that the previously reported deficits occur rather late in the disease. From this perspective, it is clear that CRF-IR is the more susceptible of the 2 peptidergic markers in early AD. Patients with CDR = 1.0 have significantly lowered CRF-IR across all brain regions studied compared with normal controls. In contrast, only the CDR 4.0 to 5.0 group had significantly lower SLI than normal controls.

A significant correlation existed between CDR score and CRF-IR concentration. The correlation occurs with or without the inclusion of patients with end-stage CDR 4.0 to 5.0 disease. In contrast, the correlation of SLI with CDR was totally dependent on the presence of the CDR 4.0 to 5.0 group. Furthermore, although SLI levels were significantly correlated with CDR, their inclusion in multiple regression analyses did not add significantly to the correlation observed with CRF-IR alone. Correlations between neuropathologic indices and CRF-IR were significant but required the inclusion of patients with CDR 4.0 to 5.0. A similar circumstance held for SLI. Without CDR 4.0 to 5.0 patients, there was no correlation between neuropathologic indices and SLI.

Close scrutiny of the results of the CDR 0.5 group permits consideration of whether either peptide could be a very early marker of AD. The possibility that CRF-IR may be decreased with the onset of the earliest symptoms of AD would be suggested if patients with no AD pathologic lesions in the CDR 0.5 group had higher CRF-IR levels than those with some AD neuropathologic lesions. The 4 patients with no AD pathologic lesions had 4 of the 8 highest CRF-IR concentrations. With the elimination of those patients, the distribution of CRF-IR concentrations in the CDR 0.5 group became similar to the CDR 1.0 group and, therefore, possibly lower than normal controls. It is less clear whether SLI would be as robust an early marker. Among the 6 highest SLI concentrations, 2 derive from patients with no AD pathologic lesions. Elimination of those 2 data points did not change the distribution of SLI concentrations in the CDR 0.5 group.

Thus, decreases in CRF-IR concentrations, but not SLI, are apparently present in early AD. The failure of SLI levels to decrease in mild and moderate dementia is reminiscent of the result obtained with cholinergic markers.30 Perhaps if cholinergic or somatostatinergic neurons are involved in early AD, there are compensatory mechanisms in these neurons, or their neighbors, that produce increases in markers of these neurotransmitters or neuromodulatory systems. In the case of neurons containing CRF, compensation to early injury may be at a postsynaptic level, since it has been demonstrated that CRF receptors are up-regulated in AD.17 In contrast, muscarinic receptors and somatostatinergic receptors have not been so consistently found to be increased in patients with AD.34

Recently, it has been reported that CRF can be substantially protein bound in the brain, and only free CRF
is active. This study measured total CRF, regardless of binding. Conceivably, the differences found would be even more apparent if only free CRF were measured. In that circumstance, free CRF would have been an even more robust marker than was found in the current investigation. Such a possibility should encourage further studies of CRF in cerebrospinal fluid of patients with AD in which correlations with the severity of AD have been reported.

Throughout this study, we have referred to our patients with mild dementia, those in the CDR 0.0 to 2.0 groups, as representing cases of early or mild AD. We have equated early dementia to early AD because we specifically excluded from the study cohort those patients who had non-AD–related neuropathologic lesions. Since many of these patients with mild dementia did not have sufficient densities of AD-related pathologic lesions to meet criteria for AD (Table 1), it is conceivable that they may have either remained mildly demented and neuropatho-

Table 2. Pearson Product Moment Correlation of Corticotropin-Releasing Factor Immunoreactivity (CRF) and Somatostatin-like Immunoreactivity (SLI) in the 9 Cortical Regions With Clinical Dementia Rating Scale (CDR)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>CRF*</th>
<th>SLI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle frontal gyrus (Brodmann area 8)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Anterior cingulate gyrus (Brodmann area 32)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Inferior frontal gyrus (Brodmann area 44)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Superior temporal gyrus (Brodmann area 22)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Middle temporal gyrus (Brodmann area 21)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Inferior temporal gyrus (Brodmann area 20)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Entorhinal cortex (Brodmann area 36/38)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Inferior parietal lobule (Brodmann area 7)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
<tr>
<td>Occipital cortex (Brodmann area 17)</td>
<td>r, Including CDR 4.0-5.0 Group</td>
<td>r, Excluding CDR 4.0-5.0 Group</td>
</tr>
</tbody>
</table>

* Asterisks indicate P < .05 after Bonferroni correction.
logically equivocal with respect to diagnosis or progressed to some non-AD neuropathologic disease had they lived longer. It is therefore necessary to keep in mind that, although the most parsimonious assumption is that these patients with mild dementia represent cases of early or mild AD, some may have had dementia due to a different, unidentified neuropathologic process. Thus, the results of this study should be viewed as providing direct evidence for the involvement of CRF-IR and SLI in different stages of dementia but only indirect evidence for CRF-IR and SLI involvement at different stages of AD.

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