CHOLINESTERASE ACTIVITIES IN CEREBROSPINAL FLUID OF PATIENTS WITH SENILE DEMENTIA OF ALZHEIMER TYPE

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SUMMARY

Acetylcholinesterase (AChE) and nonspecific cholinesterase (nsChE) activities of lumbar cerebrospinal fluid (CSF) from patients with a clinical or histological diagnosis of Alzheimer's disease have been compared with those of normal age-matched control patients and patients with dementia of non-Alzheimer aetiology. No significant differences in the AChE activity of lumbar CSF from histologically and clinically diagnosed Alzheimer's disease patients and normal age-matched controls were found, although they could be distinguished from controls and other dementias by their lower lumbar CSF levels of nsChE activity and by their elevated ratio of AChE/nsChE. A lower level of AChE activity was observed in the lumbar CSF of patients with dementia of non-Alzheimer aetiology.

The AChE and nsChE activities of ventricular CSF obtained at postmortem have also been examined. The AChE activity of the ventricular CSF of patients with histologically confirmed Alzheimer's disease was 66\% lower than that of age-matched controls; these patients could also be distinguished from normals by their lower levels of nsChE and by the elevated ratio of AChE/nsChE activities. A molecular defect in the AChE in the ventricular CSF of Alzheimer patients is indicated by the finding that the enzyme failed to show inhibition by high concentrations of substrate.

The lower level of AChE in ventricular CSF may reflect the changes in this enzyme in forebrain regions of Alzheimer patients. Although it is at present not possible to correlate the lower levels of nsChE found in CSF with any known brain pathology, the significantly altered ratio of AChE/nsChE activities in lumbar CSF may possibly form the basis for a diagnostic test of Alzheimer type dementia.

INTRODUCTION

Biochemical and histochemical studies have demonstrated a widespread deficit in the activity of acetylcholinesterase (AChE) in the brains of patients with senile dementia of the Alzheimer type (SDAT) at postmortem (see Hardy et al., 1985, for review). The extent of these reductions in AChE activity was found to be correlated with the severity of the dementia at death, that is, with the number of
neuropathological abnormalities and with the mental test score of the patients (Perry et al., 1978). It has been noted that all the cell groups outside the cortex in which the typical pathological abnormalities, such as cell loss and/or plaques and tangles are found in Alzheimer’s disease, contain AChE (Smith and Cuello, 1984), and these authors have suggested that the disease is connected with an abnormality of some of the cells that contain AChE. Consistent with such an idea is the finding that cortical AChE from patients with SDAT exhibits abnormal kinetic properties and is no longer subject to substrate inhibition, unlike that from normal age-matched controls (Perry and Perry, 1981). The plaques which are a feature of the disease have been shown to stain positively for AChE (Perry et al., 1980; Perry and Perry, 1981), indicating that the neurites and terminals forming the plague (Probst et al., 1983) may be derived from AChE-containing cells. Indeed, AChE-staining neurite-like structures have been identified in the plaques of elderly monkeys (Struble et al., 1982).

One soluble isoenzyme of AChE is secreted from certain brain regions of animals on electrical and drug stimulation (Greenfield, 1984) and AChE is present in human cerebrospinal fluid (CSF) (Tower and McEachern, 1949). It might therefore be predicted that low levels of brain tissue AChE would be reflected in lower levels of AChE in the CSF. However, studies of AChE activity in lumbar CSF from patients with dementia have produced conflicting results; several authors report a significant decrease in the AChE activity of patients with SDAT (Soininen et al., 1981; Arendt et al., 1984; Tune et al., 1985), whereas other authors (Davies, 1979; Wood et al., 1982; Deutsch et al., 1983; Lai et al., 1984) report no change, or a slight tendency for an increase (Johnson and Domino, 1971) in levels of the enzyme in lumbar CSF. A wide variety of assay conditions and techniques have been employed in these studies, with only one of them (Arendt et al., 1984) using the specific AChE inhibitor BW284c51 to distinguish between ‘true’ acetylcholinesterase and nonspecific cholinesterase in all their samples. Thus most studies actually report total cholinesterase activity, rather than AChE activity, as they claim. The main criticism of the above studies is that (with the exception of the study by Arendt et al., 1984) diagnosis of Alzheimer’s disease was based mainly on clinical criteria, and rarely confirmed by postmortem or histological examination. A definite diagnosis of Alzheimer’s disease is only possible if the neuropathology of the disease is demonstrated in samples of brain material, that is the presence of senile plaques and neurofibrillary tangles. In the present work, the ‘true’ AChE activities of lumbar CSF from patients who have had the clinical diagnosis of Alzheimer’s disease confirmed by cortical biopsy have been examined and compared with those of age-matched controls. The results, which are reported in this paper, have been compared with those from a study of lumbar CSF AChE activity in patients with a purely clinical diagnosis of Alzheimer’s disease using strict criteria. The AChE activity of ventricular CSF obtained at postmortem has also been examined in patients with a histological diagnosis of Alzheimer’s disease. A brief report of some of the results from the latter study has been published (Appleyard et al., 1983).
all cases, patients with dementia of non-Alzheimer aetiology and nondemented controls were also studied.

In view of the reported alterations in the kinetic properties of AChE in cortical homogenates from patients with SDAT (Perry and Perry, 1981), the CSF samples have been examined to see if AChE is inhibited by high substrate concentrations in patients with the disease.

The activities of AChE and butyrylcholinesterase (BuChE) are altered in opposite directions in the brain (Perry et al., 1978) and plasma (Smith et al., 1982) of patients with Alzheimer's disease, and this might be reflected in a decrease in the ratio of AChE/BuChE in the CSF of patients with the disease. Indeed, Arendt et al. (1984) found such a decrease in their series of histologically-diagnosed patients. The AChE/BuChE ratios have therefore been examined in the three series of patients reported in this paper.

METHODS

Patients

1. Clinical diagnosis only. Eleven patients with senile dementia of the Alzheimer type (SDAT), 8 patients with dementia of uncertain aetiology and 15 controls with no evidence of dementia or other neurological disease were studied. All patients were assessed prospectively and diagnosed on the basis of their performance on an abbreviated Mental Test Score (Hodkinson, 1972) and the modified Kew Test (McDonald, 1969; Hare, 1978) and by an activity of daily living assessment. For all SDAT patients all other possible causes of dementia were excluded by laboratory tests, the Hachinski Score (Hachinski et al., 1975) and in 6 patients by CT scan. Patients in the 'mixed' group included 4 patients with clinical evidence of multi-infarct dementia and 3 patients with a history of progressive dementia that could be attributed to other factors (recent Herpes zoster, prolonged haemodialysis for membranous glomerulonephritis or serological evidence of treponemal disease). A further patient was shown to have a depressive illness with memory disturbance improving markedly, although not to normal, with antidepressant treatment (pseudodementia). All patients in the control group complained of low back pain with or without sciatica, and were attending for myelography. In all patients myelography was either normal or revealed evidence of a disc lesion without evidence of a spinal block. None had a history of memory disturbance.

Two SDAT patients were receiving tricyclic antidepressants and 1 a phenothiazine while in the mixed group one was receiving L-DOPA and anticholinergic drugs, 1 was receiving carbamazepine and another prednisolone. Control patients were not receiving psychoactive drugs other than mild hypnotics.

CSF was obtained after 1–2 h of recumbency using a standard lumbar puncture technique, and sent for microbiological, serological and biochemical analysis as clinically indicated. After discussion with relatives, where necessary, informed consent for the removal of an additional 1–2 ml for acetylcholinesterase estimation was obtained for all individuals. Samples were kept at 4° C until centrifugation, and stored at –70° C until analysis. All samples were clear and free of blood.

2. Clinical diagnosis with histological confirmation at brain biopsy. Seven patients with senile dementia of the Alzheimer type, 4 patients with dementia of uncertain aetiology, and 6 controls with no evidence of dementia or other neurological diseases were studied. The procedure of cerebral biopsy was approved by the Manchester Central District Ethical Committee.

All patients were assessed for cognitive impairment shortly before surgery (see Neary et al., 1986). Patients in the SDAT group all had a clinical diagnosis of Alzheimer's disease and showed the characteristic histopathological abnormalities (plaques and tangles) in neocortical tissue removed at
diagnostic craniotomy. Patients in the non-SDAT group had a clinical diagnosis of probable SDAT but did not display the histological changes that are characteristic of the disease upon examination of neocortical biopsy samples. Patients in the control group were all undergoing investigation for a variety of peripheral and spinal complaints.

CSF (0.5–5.0 ml) was removed before operation from the lumbar subarachnoid space, following overnight fast, and frozen at −70°C until analysis. All patients were drug free at the time of the lumbar puncture.

3. Postmortem diagnosis of Alzheimer’s disease. Ventricular CSF obtained at postmortem on separate groups of demented and intellectually unimpaired elderly subjects was studied. All patients were assessed prospectively during life using the same techniques described in Section 1. Blood-free samples were stored at −70°C until analysis and centrifuged prior to assay.

The ages (range 67–100 yrs), postmortem delay (range 0–4 days) and storage time before assay were similar for all groups of patients. The medication of the Alzheimer’s disease and control patients was similar with the most frequently prescribed drugs being phenothiazines, butyrophenones and tricyclic antidepressants.

Biochemical analysis

All biochemical analyses were performed without prior knowledge of the diagnosis.

Acetylcholinesterase activity was measured at $V_{ma}$, by a modified version (Chubb and Smith, 1975) of the method of Ellman et al. (1961). Acetylthiocholine (1.0 mM) was used as substrate and the assay was performed in 0.05 M Na/K phosphate buffer, pH 7.0 at 30°C. In order to distinguish between AChE and nonspecific cholinesterase activity assays were performed both in the presence and absence of the specific AChE inhibitor BW284c51 (1.5 x 10$^{-5}$ M).

The assay conditions described above allow an estimation of the nonspecific cholinesterase activity present in the samples, but do not provide an accurate quantitative measure of butyrylcholinesterase activity, since the substrate concentration used is suboptimal for this enzyme. BuChE activity was also measured by the modified version of the Ellman method using 1.5 x 10$^{-2}$ M butyrylthiocholine as substrate, which is optimum for this enzyme but is not a substrate for AChE.

Estimation of the protein content of the CSF was performed by the method of Lowry et al. (1951), following precipitation by 6% trichloroacetic acid.

All samples of a sufficient volume were assayed for AChE activity at various substrate concentrations from 0.1 to 20 mM acetylthiocholine to determine whether the enzyme showed substrate inhibition. Only a limited volume of ventricular CSF was available for study. In this case 19 control samples and 9 samples of CSF from patients with SDAT were analysed to determine whether the AChE was subject to substrate inhibition by comparing the activities of the samples at substrate concentrations of 1 mM and 20 mM acetylthiocholine. These substrate concentrations were chosen after examination of the complete substrate curves obtained for 6 control samples; peak AChE activity occurred at substrate concentrations of 1 to 2 mM acetylthiocholine whilst marked inhibition of activity was observed at 20 mM acetylthiocholine.

Statistical analysis

Data are presented as the mean ± SEM. Results were analysed by Student’s t test and by the Spearman rank correlation test.

RESULTS

Lumbar CSF from patients with a purely clinical diagnosis

The mean AChE activity of lumbar CSF, expressed both per ml CSF and per mg protein, from patients with clinical dementia of non-Alzheimer aetiology was
markedly lower than in age-matched controls, whereas control levels were observed in patients with SDAT (Table 1, fig. 1).

The mean nsChE activity (measured with a substrate of 1.0 mM acetylthiocholine) in CSF from patients with SDAT was significantly lower than in controls (Table 1, fig. 1). However, when the Alzheimer's patients were subclassified according to the severity of the dementia, as determined by their performance on the Kew Test, there was no difference in the nsChE activity from patients with severe dementia compared with that in patients with mild dementia. The patients with dementia of non-Alzheimer aetiology had nsChE activities in their CSF that were not significantly different from those observed in normal aged subjects (Table 1).

Table 1. Cholinesterase activities and protein content of lumbar CSF from patients with a clinical diagnosis of dementia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Ages (yrs)</th>
<th>AChE (nmol/min/ml)</th>
<th>nsChE (nmol/min/ml)</th>
<th>Protein (g/l)</th>
<th>SA AChE</th>
<th>AChE/BuChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (&lt; 50 yrs, n = 4)</td>
<td>37.0 ± 1.7</td>
<td>13.5 ± 1.8</td>
<td>3.2 ± 0.4*</td>
<td>0.33 ± 0.05</td>
<td>43.8 ± 9.5</td>
<td>1.79 ± 0.21*</td>
</tr>
<tr>
<td>Control (&gt; 50 yrs, n = 12)</td>
<td>59.3 ± 2.1</td>
<td>19.2 ± 2.3</td>
<td>5.2 ± 0.6</td>
<td>0.42 ± 0.06</td>
<td>43.8 ± 9.5</td>
<td>0.88 ± 0.10</td>
</tr>
<tr>
<td>SDAT (n = 11)</td>
<td>68.9 ± 2.0</td>
<td>16.9 ± 1.3</td>
<td>3.42 ± 0.5*</td>
<td>0.33 ± 0.03</td>
<td>55.2 ± 9.9</td>
<td>1.34 ± 0.15*</td>
</tr>
<tr>
<td>Non-SDAT dementia (n = 6)</td>
<td>76.0 ± 3.9</td>
<td>10.4 ± 1.6*</td>
<td>6.8 ± 3.5</td>
<td>0.57 ± 0.26</td>
<td>28.1 ± 6.1*</td>
<td>0.82 ± 0.13</td>
</tr>
<tr>
<td>Pseudodementia (n = 1)</td>
<td>77.0</td>
<td>15.4</td>
<td>1.94</td>
<td>0.225</td>
<td>68.4</td>
<td>2.56</td>
</tr>
</tbody>
</table>

1Acetylcholinesterase (AChE), nonspecific cholinesterase (nsChE) activities are expressed as nmol acetylthiocholine hydrolyzed/min/ml CSF, butyrylcholinesterase (BuChE) activity is expressed as nmol butyrylthiocholine hydrolysed/min/ml CSF Specific activity (SA) AChE is expressed per mg protein. *, ** Significantly different from controls (> 50 yrs) with P < 0.05 and P < 0.005, respectively.

A similar situation was also observed with BuChE activity (measured with 1.5 x 10^-2 M butyrylthiocholine as substrate). BuChE activity of CSF from cases with SDAT ranged from 3.82 to 26.3 mU/ml, and the mean value (13.6) was significantly (P < 0.025) lower (by 35%) than that for controls (mean, 20.8; range 11.3 to 35.4 mU/ml).

The ratio of AChE activity to BuChE activity or to nsChE activity in the patients with SDAT was significantly elevated over the ratio observed in control (> 50 yrs old) patients; this ratio was unchanged in patients with dementia of non-Alzheimer aetiology (Table 1, fig. 1).

Age-related changes in AChE, nsChE and ratio AChE/BuChE were also observed.

Lumbar CSF from patients with a biopsy-confirmed diagnosis

The results obtained in this group of patients were similar to those observed in the patients with a purely clinical diagnosis. The AChE activity of CSF, expressed per ml CSF and per mg protein, from patients with dementia of non-Alzheimer aetiology tended to be lower than those in the CSF from age-matched controls; in
FIG. 1. Cholinesterase activities in lumbar and ventricular CSF from patients with a diagnosis of Alzheimer's disease. Clinical group = patients with a purely clinical diagnosis of Alzheimer's disease; biopsy group = clinical diagnosis confirmed by histological examination at biopsy; in both groups lumbar CSF was examined. Postmortem group = ventricular CSF obtained at postmortem from patients with a histological diagnosis of Alzheimer's disease. Open columns = normal age-matched control patients with no evidence of neurological disease; cross-hatched columns = Alzheimer's disease patients. Acetylcholinesterase (A) and nonspecific cholinesterase (B) activities are expressed as nmol acetylthiocholine hydrolyzed/min/ml CSF. Specific activity acetylcholinesterase (C) is expressed as nmol acetylthiocholine hydrolysed/min/mg protein. *, ** Significantly different from control with \( P < 0.05 \) and \( P < 0.005 \), respectively.

those with SDAT the levels were normal (Table 2, fig. 1). Nonspecific cholinesterase activity (measured with a substrate of 1.0 mM acetylthiocholine) was markedly lower than controls in the patients with SDAT, but unchanged in the patients with dementia of non-Alzheimer aetiology (Table 2).

The ratio of AChE activity to BuChE activity or to nsChE activity in the patients with SDAT tended to be higher than that observed in normal control patients (Table 2, fig. 1), and there was a significant correlation between this ratio and the
Acetylcholinesterase (AChE) and nonspecific cholinesterase (nsChE) activities are expressed as nmol acetylthiocholine hydrolysed/min/ml CSF; specific activity acetylcholinesterase is expressed per mg protein; protein content is expressed as mg/ml CSF

* Significantly different from controls with \( P < 0.05 \). \( ^* \) Significantly different from controls with \( P < 0.05 \) by the Wilcoxon test.

### Table 2. Cholinesterase Activities and Protein Content of Lumbar CSF from Patients with a Histological Diagnosis (from Cortical Biopsy) of Alzheimer's Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AChE</th>
<th>nsChE</th>
<th>Protein</th>
<th>SA AChE</th>
<th>AChE/ChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 6)</td>
<td>8.44±1.75</td>
<td>1.32±0.48</td>
<td>0.18±0.03</td>
<td>50.3±7.1</td>
<td>6.81±1.42</td>
</tr>
<tr>
<td>SDAT (n = 7)</td>
<td>6.32±1.11</td>
<td>0.48±0.18*</td>
<td>0.12±0.02</td>
<td>52.1±7.2</td>
<td>17.3±7.7</td>
</tr>
<tr>
<td>Non-SDAT dementia (n = 4)</td>
<td>4.43±0.06*</td>
<td>0.91±0.20</td>
<td>0.17±0.03</td>
<td>28.9±5.8</td>
<td>5.45±1.09</td>
</tr>
</tbody>
</table>

The cognitive impairment rating of the SDAT patients with a correlation coefficient of 0.7590. The ratio in patients with non-SDAT dementia however, was not different from that in control patients (Table 2).

**Ventricular CSF obtained at postmortem**

The mean AChE activity in ventricular CSF obtained at autopsy from SDAT patients was markedly lower than in controls (Table 3, fig. 1). Two patients were diagnosed clinically as having mild Alzheimer's disease and showed normal cortical ChAT levels at postmortem examination. The AChE activities of the CSF from these patients were in the normal range (13.4 and 14.7 mU/ml). However, there was no correlation between the AChE activities of the CSF samples from SDAT cases with the severity of the dementia as determined by cortical plaque and tangle counts. The AChE activities of ventricular CSF from patients with other neurological diseases, including non-Alzheimer dementia, Parkinson's disease and depression, were not significantly different from control levels (see Table 1).

The mean nsChE activity in CSF from patients with Alzheimer's disease was also lower than in controls (Table 3, fig. 1) and again the nsChE activities of the CSF from the 2 patients with mild SDAT were in the normal range (2.36 and 9.73 mU/ml). The nsChE activities of ventricular CSF from patients with Parkinson's disease, non-Alzheimer dementia or depression were not significantly different from control levels (see Table 3), although the nsChE activity from patients who had recently suffered from cerebrovascular lesions (strokes) was elevated over control levels. BuChE activities were not measured due to lack of sample.

In the ventricular CSF samples from patients with SDAT there was a significant correlation \( (P < 0.02) \) between AChE and nsChE activities \( (r = 0.723) \). The ratio of AChE to nsChE activity in the CSF from patients with SDAT was higher than that of control patients (Table 3, fig. 1), whereas the AChE/nsChE ratios from the patients with other neurological diseases were in the control range (Table 3).

The mean TCA-precipitable protein content was significantly lower in the SDAT patients; again the values for the 2 patients with mild SDAT were in the normal range (0.98 and 1.06 mg/ml). The protein contents of CSF samples from patients...
TABLE 3. CHOLINESTERASE ACTIVITIES AND PROTEIN CONTENT OF VENTRICULAR CSF OBTAINED AT POSTMORTEM FROM PATIENTS WITH DEMENTIA AND VARIOUS OTHER NEUROLOGICAL DISEASES

<table>
<thead>
<tr>
<th>Disease</th>
<th>Controls (n=23)</th>
<th>SDAT (n=11)</th>
<th>Parkinson's disease (n=6)</th>
<th>PD/dementia (n=5)</th>
<th>Non-SDAT dementia (n=5)</th>
<th>Strokes (n=10)</th>
<th>Other neurological diseases (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>83.2 ± 1.9</td>
<td>81.2 ± 4.7</td>
<td>78.0 ± 3.7</td>
<td>82.0 ± 2.5</td>
<td>81.2 ± 1.4</td>
<td>81.6 ± 2.8</td>
<td>76.0</td>
</tr>
<tr>
<td>AChE (nmol)</td>
<td>16.7 ± 2.6</td>
<td>5.71 ± 1.37**</td>
<td>10.9 ± 2.8</td>
<td>18.2 ± 5.5</td>
<td>21.3 ± 3.6</td>
<td>19.8 ± 5.1</td>
<td>24.7</td>
</tr>
<tr>
<td>nsChE (nmol)</td>
<td>12.4 ± 1.6</td>
<td>3.52 ± 1.16**</td>
<td>12.9 ± 4.9</td>
<td>21.3 ± 8.6</td>
<td>10.8 ± 0.73</td>
<td>24.4 ± 4.1</td>
<td>26.5</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>1.31 ± 0.17</td>
<td>0.66 ± 0.13*</td>
<td>1.24 ± 0.18</td>
<td>1.63 ± 0.21</td>
<td>1.35 ± 0.07</td>
<td>1.86 ± 0.20*</td>
<td>3.19</td>
</tr>
<tr>
<td>SA AChE (nmol)</td>
<td>13.9 ± 2.0</td>
<td>8.81 ± 1.43*</td>
<td>8.78 ± 1.94</td>
<td>10.8 ± 2.06</td>
<td>16.6 ± 3.91</td>
<td>12.4 ± 2.9</td>
<td>7.8</td>
</tr>
<tr>
<td>AChE/ChE</td>
<td>1.78 ± 0.36</td>
<td>3.42 ± 1.04*</td>
<td>1.08 ± 0.25</td>
<td>1.08 ± 0.25</td>
<td>1.99 ± 0.31</td>
<td>1.14 ± 0.26</td>
<td>1.01</td>
</tr>
</tbody>
</table>

1 Acetylcholinesterase (AChE) and nonspecific cholinesterase (nsChE) activities are expressed as nmol acetylthiocholine hydrolyzed/min/ml CSF. Specific acetylcholinesterase (SA AChE) activity is expressed per mg protein. ** Significantly different from controls with \( P < 0.05 \) and \( P < 0.005 \), respectively. (Nine of the Alzheimer patients and 14 of the control patients are the same as those in a brief early report of this work (Appleyard et al., 1983), tissue samples from these patients have been included in two previous studies (Wilcock and Earn, 1982, Wilcock et al., 1982).

with other neurological diseases were also in the control range (see Table 3). Both AChE and nsChE activities significantly correlated with the protein content in the Alzheimer’s cases (fig. 2).

Substrate inhibition of AChE

All samples of lumbar CSF obtained from patients with a clinical diagnosis exhibited profound substrate inhibition at acetylthiocholine concentrations of 20 mM, with peak activity occurring at 1 to 2 mM acetylthiocholine.

Samples of ventricular CSF obtained at postmortem were also assessed for substrate inhibition of AChE. All the control samples exhibited marked substrate inhibition at 20 mM acetylthiocholine compared to the AChE activities observed at 1 mM acetylthiocholine (ratio 20 mM/1 mM activities = 0.346 ± 0.054), while the AChE in the CSF from patients with Alzheimer’s disease failed to exhibit any such substrate inhibition with similar activities being measured at 1 mM and 20 mM acetylthiocholine (ratio 20 mM/1 mM activities = 0.947 ± 0.163). An alternative interpretation of these results is that the substrate curve of AChE is displaced in Alzheimer’s disease such that peak activity will no longer be observed at 1 mM acetylthiocholine. However, enough sample volume was available from 3 patients to allow substrate curves for AChE activity at various concentrations of acetylthiocholine from 0.1 to 20 mM to be performed. These samples failed to exhibit inhibition over this range of substrate concentrations.
DISCUSSION

Cholinesterases and dementia

The results obtained in this study indicate that the levels of cholinesterases within CSF are abnormal in demented patients. Acetylcholinesterase activity is reduced in ventricular CSF of patients exhibiting the clinical and histological features of Alzheimer's disease and in the lumbar CSF of patients with dementia of non-Alzheimer aetiology. A pronounced reduction in nsChE activity and an altered ratio of AChE/nsChE activity also occurs in ventricular and lumbar CSF of SDAT patients.

These biochemical abnormalities are not artefacts arising from drug treatment. Only 27% of the clinically diagnosed patients in the lumbar CSF study were on psychoactive drugs, and only one of these was receiving an antipsychotic phenothiazine drug, whereas all the histologically diagnosed patients in the lumbar CSF study were drug-free. Drug treatment also seems an unlikely explanation for the lower AChE levels observed in the ventricular CSF of patients with Alzheimer's disease as the medication for both these patients and the control group was not very different. For example the mean AChE level of the 3 patients in the control group who were receiving antipsychotic drugs was 21.5 mU/ml compared with a mean level of 4.3 mU/ml in the 3 SDAT patients who were receiving similar drugs.

There is a possibility that the apparent decreases in the AChE activity of ventricular CSF in SDAT patients is a postmortem artefact since many Alzheimer's disease patients die in a cachetic state (Hardy et al., 1985a) and it is difficult to select adequate control groups for this particular variable. A prolonged agonal phase before death is known to cause biochemical changes in the brain (Hardy et al., 1985b) which could presumably be reflected in the CSF. However, it is unlikely in the present study that the differences are due to postmortem delay or storage time before assay since these were similar for all the groups and did not correlate with the levels of AChE, nsChE or protein. It would therefore appear that the alterations in cholinesterase levels of CSF from demented patients are a real feature of the disease process itself.

Possible relevance of CSF acetylcholinesterase to the disease process

A markedly lower AChE activity of ventricular CSF was observed in patients dying with Alzheimer's disease. This phenomenon was restricted to those patients with Alzheimer's disease, with normal levels of AChE being observed in patients with dementia of non-Alzheimer aetiology, Parkinson's disease and depression. Despite the lack of a significant correlation between the decrease in AChE activity and the severity of the dementia, as determined by cortical plaque and tangle counts, it does appear to be related to the disease process as the 2 patients with clinically mild dementia and normal cortical ChAT levels did not exhibit a deficit of AChE activity in their CSF.

The protein content of ventricular CSF followed a similar pattern so that it was
significantly correlated with the AChE activity in SDAT patients (fig. 2), and the mean specific activity of AChE (expressed per mg protein) was below that of controls (Table 3). It is possible that these decreases in AChE activity and protein content are the result of dilution of the CSF due to the enlarged ventricular volume of SDAT patients (De Leon et al., 1980; Soininen et al., 1982). Alternatively, the lowered protein content of the ventricular CSF could be a consequence of the reported decreased protein synthetic ability of nerve cells in Alzheimers' disease (Mann et al., 1981); indeed reductions in specific brain proteins such as soluble tubulin have been reported in SDAT patients (Kosik et al., 1982; Borthwick et al., 1985). If the latter is the case, then the lowered levels of AChE in ventricular CSF that are observed in the SDAT patients are the result of reduced secretion from the brain. The AChE activity of ventricular CSF is presumably derived from those areas of the brain that are close to the ventricular space, including the cortex and hippocampus, both of which are profoundly affected in Alzheimer's disease (but not in other dementias; Perry et al., 1978) with widespread decreases in AChE activity occurring in all cortical and hippocampal regions (Davies, 1979). Acetylcholinesterase is secreted from the rat hippocampus (Appleyard and Smith, 1985), and this secretion would presumably also be decreased in SDAT patients, resulting in a lowered level of AChE in ventricular CSF. In addition, the form of AChE secreted from the brain is abnormal in patients with SDAT and is no longer subject to substrate inhibition which must have profound consequences on the function of the enzyme.
In contrast, normal levels of AChE, exhibiting normal kinetic behaviour, were observed in lumbar CSF of SDAT patients. In animal studies the AChE activity of cisternal CSF is higher than that of ventricular CSF (Greenfield, 1977) suggesting that brain structures caudal to the ventricular system make a significant contribution to the secreted AChE present in cisternal CSF. A similar situation exists in humans: the lack of a rostrocaudal gradient of AChE activity indicates a diffuse origin of the AChE in lumbar CSF, including the spinal cord (Johnson and Domino, 1971; Lal et al., 1984). Contributions from these regions to the AChE in lumbar CSF could therefore mask any changes occurring in ventricular CSF. Acetylcholinesterase is widely distributed in human spinal cord and is present in cholinergic motor neurons (Houser et al., 1983; Barber et al., 1984). However, to date no studies have examined levels of AChE and ChAT in the spinal cord of SDAT patients, although it has been reported that motor neurons are unaffected in Alzheimers' disease (Davies, 1983; Hardy et al., 1985a). The fact that the AChE activity of lumbar CSF is normal suggests that the spinal cord is not affected by this disease. This is probably not the case in dementia of non-Alzheimer aetiology since such patients exhibited decreased levels of AChE activity in lumbar, but not ventricular, CSF indicating decreased secretion of the enzyme from the spinal cord.

*Nonspecific cholinesterase/butyrylcholinesterase activities of CSF from alzheimer’s disease patients*

The only consistent change found in all SDAT patients studied was a lower level of nsChE and BuChE activity in the CSF. This parameter also distinguished the SDAT patients from those with other dementias (except pseudodementia) since no decreases were observed in the CSF of these patients. This phenomenon is also responsible for an increased ratio of AChE/nsChE or AChE/BuChE activities in all the SDAT patients, even in ventricular CSF, and again this effect is specific for Alzheimer’s disease (and pseudodementia).

Butyrylcholinesterase is present in the brain (see Silver, 1974) and biochemical studies have demonstrated that the regional variation of BuChE activity is less than that of AChE with comparatively higher levels of BuChE activity occurring in white matter (Cavanagh et al., 1954). Histochemical studies have shown that BuChE is mainly located in glial cells (Koelle, 1954), but it is also present in nerve cells (e.g., Graybiel et al., 1982) with exceptionally high activity being observed in a few selected nuclei, which are species-dependent (Friede, 1967). In man BuChE is present in neurons in the subependymal tissue near the nucleus of the solitary tract, the dorsal vagus nucleus and the tissue around the central spinal canal. However, very little BuChE is observed in the cerebral cortex and hippocampus, areas in which increased level of BuChE has been reported in Alzheimer’s disease (Perry et al., 1978).

No studies have yet been performed to determine whether BuChE can be secreted from these brain regions, or from glial cells, and the relationship between the decreased levels of BuChE in lumbar CSF and the increased brain tissue levels of
SDAT patients is therefore uncertain. The function of BuChE within the brain is not yet known (see Silver, 1974; Kutty, 1980) and the significance of these alterations in BuChE activity in the disease process is unclear. Despite these uncertainties, however, it would appear that estimation of BuChE activity and/or the AChE/BuChE ratio in lumbar CSF may prove a valuable diagnostic aid in Alzheimer's disease.

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Note added in proof. Similar changes in the BuChE content of lumbar CSF in Alzheimer’s disease 
have been reported by Heff et al. (1986) _Journal of the Neurological Sciences_, 72, 121–129.

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