Cholinesterase Activity in Plasma, Erythrocytes, and Cerebrospinal Fluid of Patients with Dementia of the Alzheimer Type

J. K. Marquis, L. Volicer, K. A. Mark, L. K. Direnfeld, and M. Freedman

Cholinesterases, including pseudocholinesterase (BChE) of human plasma and acetylcholinesterase (AChE) of erythrocytes and cerebrospinal fluid (CSF), have been considered as possible markers in dementia of the Alzheimer type (DAT). Reported data, however, are widely varied, and no significant pattern emerges when total enzyme activity is assayed. In the present studies, we have reexamined the relationship of ChE activities in DAT and control patients. ChE activity was measured in plasma, erythrocytes, and CSF from DAT patients and compared with normal controls as well as with samples from patients with a diagnosis other than DAT. Early age onset (presenile) and late age onset (senile) DAT were also compared. No significant differences in total enzyme activity were found in any of the comparisons. Calculations of AChE/BChE ratios in CSF also provided no significant indication of any changes in ChE activities in DAT. It is suggested that measurements of total AChE or BChE activity in these biological materials do not provide a useful index of alterations in central cholinergic function in patients with DAT.

Introduction

Cholinesterases, including both the true acetylcholinesterase (AChE) and pseudocholinesterase or butyrylcholinesterase (BChE), have been considered as possible markers in dementia of the Alzheimer type (DAT), i.e., as indicators of altered cholinergic function in the Alzheimer brain. Clinical data reported to date, however, are widely varied, with no significant pattern emerging from a review of recent studies. It has been suggested, for example, that total cerebrospinal fluid (CSF) AChE is decreased in DAT (Soininen et al. 1981), but this finding was not confirmed in later studies (Wood et al. 1982; Deutsch

From the Department of Pharmacology, Boston University School of Medicine (J.K.M.); the E. N. Rogers Memorial Veterans Hospital, Geriatric Research Education Clinical Center, Bedford, MA (L.V., K.A.M.); and the Department of Neurology, Veterans Administration Medical Center, Boston, MA (L.K.D., M.F.).

Supported by a grant from the Center for Brain Sciences and Metabolism Charitable Trust, Cambridge, MA, and by funds from the Veterans Administration.

Address reprint requests to Dr. Judith K. Marquis, Dept. of Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

Received August 22, 1984; revised December 1, 1984.

0006-3223/85/$0.00

et al. 1983). Smith et al. (1982) reported a marked increase in plasma BChE and a slight decrease in erythrocyte AChE in DAT compared with age-matched controls. Perry et al. (1982) found no change in plasma AChE compared with age-matched controls. It has also been suggested that the ratio of AChE to BChE is decreased in the CSF of DAT patients (Arendt et al. 1984), and it has been reported that total AChE activity is decreased in post mortem samples of intraventricular fluid from DAT patients (Appleyard et al. 1983).

In the present studies, we measured true AChE and total BChE in CSF, serum, and erythrocytes from DAT patients and compared the results with data from normal controls and from patients with a diagnosis other than DAT. Early age onset (presenile) and late age onset (senile) DAT were also compared. CSF samples were also examined for a possible AChE gradient, and the ratios of AChE to BChE in CSF samples from DAT patients were compared with the ratios in normal controls.

The results of this study were presented in January 1984 at the third meeting of the International Study Group on Aging and Alzheimer's Disease, Zurich, Switzerland.

Methods

Thirty patients were included in the cerebrospinal fluid study and 43 in the blood study. Patients who were able to comprehend the experimental procedure gave informed consent to participate in the study after the experimental procedure was fully explained to them. In the case of demented patients, the procedure was explained to their guardians, who gave informed consent, and the subjects themselves were asked for an assent. No subjects had a history of psychiatric illness. All subjects had normal complete blood count, urinalysis, and SMA12 (initial screening laboratory tests). The control group consisted of patients with normal mental status who underwent lumbar puncture for spinal anesthesia during a urological procedure or for a myelographic procedure. The patients were fasting from midnight the night before the procedure and almost all were premedicated with meperidine HCl approximately 1 hr prior to the procedure. The DAT group included patients who fulfilled DSM-III criteria for dementia, with a mild or moderate dementia, who had no history of stroke or alcoholism, and no focal neurological signs. The average Mini-mental Score (Folstein et al. 1975) of DAT patients ranged from 0 to 25. The Parkinson's disease (PD) group included patients with mild to moderate severity of the disease.

Dopaminergic medications in PD patients were discontinued 48 hr before lumbar puncture, but the anticholinergic medications were not discontinued. The average Mini-mental Score (Folstein et al. 1975) of PD patients was 24.0 ± 5.0. Ten DAT patients with mild dementia (Mini-mental Score of 23.3 ± 3.6), 10 PD patients, and 12 controls were tested by a neuropsychological test battery designed to examine four cognitive domains: memory, visuospatial function, language, and mental control. There were no significant quantitative differences in performance between DAT and PD groups in any domain. Compared with controls, the DAT patients performed more poorly in memory, visuospatial functions, and mental control (Direnfeld et al. 1984). The patients with stroke or multiple sclerosis were hospitalized on the same ward as the DAT patients. The alcoholic group consisted of patients with a history of alcohol abuse who underwent lumbar puncture for spinal anesthesia during a urological procedure.

Blood was collected via a 21-gauge needle into sterile Vacu-tainer tubes containing heparin. The heparinized blood samples were then centrifuged at 3000 rpm for 10 min,

and the separated cells were washed three times with isotonic buffered saline (Scientific Products, Inc.). The cells and plasma were placed on dry ice and stored at −70°C until assayed.

CSF was collected via lumbar puncture in the lateral decubitus position. The lumbar punctures were performed between 8 AM and 11 AM. CSF from DAT and PD patients was collected in 1-ml aliquots from the 6th to the 20th ml, placed on dry ice at the bedside, and frozen immediately at −70°C until assayed. The 12th ml was analyzed for AChE. CSF from controls and alcoholics was refrigerated for up to 3 hr prior to being placed in the freezer, and an aliquot of the first 3–5 ml was analyzed for AChE. Controls demonstrated the stability of AChE in refrigerated samples for up to 8 hr.

AChE and BChE activity were assayed by the procedure of Ellman et al. (1961), which is a spectrophotometric method in which enzyme hydrolysis of acetylthiocholine (for AChE) or butyrylthiocholine (for BChE) is measured by following the increase of yellow color (λ = 412 nm) produced from thiocholine when it reacts with dithiobisnitrobenzoate (DTNB) ion. Aliquots of 150–200 μl of CSF, 10 μl of plasma, and 10 μl of hemolyzed erythrocytes (diluted 1 : 10 in water) were used for each assay. Thirty micromolar Iso-OMPA (Sigma, St. Louis, MO), a selective BChE inhibitor, was included in each assay for AChE. The measurements were done in triplicate on an LKB 4050 spectrophotometer interfaced to an Apple II computer. In order to calculate a ratio of AChE to BChE activities in CSF, AChE activity was measured as above with 0.5 m*M* acetylthiocholine as substrate in the presence and absence of 0.1 m*M* BW284C51 (a gift of Burroughs Wellcome Laboratories, England) as a selective inhibitor of AChE, and BChE activity was measured as above with 0.5 m*M* butyrylthiocholine as substrate in the presence and absence of 0.1 m*M* Iso-OMPA as a selective inhibitor of BChE. A ratio of AChE to BChE was then determined, using activity values for the two types of enzymes based on both their substrate and inhibitor specificities, rather than on substrate specificity alone. Protein assays were done on all samples by the method of Lowry et al. (1951), using 30 μl CSF, 10 μl plasma (diluted 1 : 20 in water), and 10 μl of packed erythrocytes (diluted 1 : 100 in water) for each assay.

Results and Discussion

Table 1 shows the results of AChE and protein assays in CSF samples. No significant differences were found by an analysis of variance (F = 0.63). It was also shown that there are no significant differences in specific activity of AChE in erythrocytes or plasma of cerebrovascular accident patients, multiple sclerosis, or Alzheimer patients when compared with neurologically normal controls (F = 2.20 and 0.70, respectively, Table 2). Clearly, a noninvasive, standardized test, or series of tests, that is sensitive to early neurochemical changes in DAT would be a valuable diagnostic tool. Although blood and CSF are relatively easily obtained, there is, as yet, no good measure of a cholinergic component in these tissues that serves as a reliable indicator of DAT.

The investigation of biochemical changes in CSF of DAT patients is hindered by difficulties in obtaining CSF from completely normal subjects. Our neurologically normal controls underwent lumbar puncture for spinal anesthesia, which is required for a urological procedure or for a myelographic procedure. Thus, some of them might have experienced some pain or discomfort. Most of them were also premedicated shortly before the lumbar puncture. The premedication was, however, unlikely to change the biochemistry of CSF in the lumbar sac, as it has been reported that the passage of CSF from the

Table 1. Levels of AChE Activity in Cerebrospinal Fluid of DAT Patients Compared with Controls[a]

Diagnosis	*n*	Age (years)[b]	AChE (μmol/hr/ml CSF)	Protein (mg/ml)
Controls	12	68.3 ± 2.0	1.21 ± 0.10	0.65 ± 0.06
Parkinsonism	10	67.8 ± 2.2	1.22 ± 0.13	0.73 ± 0.12
Alcoholism	7	67.1 ± 2.2	1.09 ± 0.23	0.94 ± 0.24
DAT	14	66.9 ± 1.9	1.13 ± 0.10	0.68 ± 0.12
Presenile	7	62.0 ± 0.7	1.11 ± 0.10	0.62 ± 0.12
Senile	7	76.1 ± 0.2	1.15 ± 0.19	0.73 ± 0.21

[a]Determined in the 12th ml of CSF from PD and DAT patients and from an aliquot of the first 3–5 ml in controls and alcoholics. The values are given as the mean ± standard error of triplicate assays from *n* patients. The data were compared statistically using the *t*-test for nonpaired data.

[b]Age at testing.

ventricles to the lumbar sac takes 60–90 min (Di Chiro et al. 1976). Our negative results are also strengthened by a lack of difference in CSF AChE activity between DAT patients with a mild form (average Mini-mental Score 23.3 ± 3.6, $n = 10$) and with a moderate form (Mini-mental Score 0, $n = 7$) of the disease. Similarly, there was no difference in the AChE activities in patients with a presenile form (onset before the age of 65) and a senile form of DAT. There was also no significant correlation between the AChE activity and age ($r = -0.03$).

Recently, Appleyard et al. (1983) reported markedly decreased AChE activity in post mortem samples of intraventricular fluid from Alzheimer's patients. Volicer et al. (1983) measured levels of monoamine metabolites in CSF of the same patients studied in Table 1 and found significant changes in some metabolites in the 20th ml of the total CSF collection that were not detected in the 6th or 13th ml fractions. It thus seemed advisable to determine whether or not there may be an AChE activity gradient in CSF. Triplicate enzyme assays on the 1st, 5th, 9th, 13th, and 16th ml of a single lumbar puncture from a patient with pseudotumor cerebri (PTC) and assays on two different lumbar punctures from a DAT patient detected no significant differences in AChE activity in any of the aliquots. The results are shown in Table 3.

In a related study, Arendt et al. (1984) reported a decreased ratio of AChE to BChE in the CSF of DAT patients. Following the same assay procedures described by these

Table 2. Levels of Cholinesterase in Plasma and Erythrocytes of DAT Patients Compared with Controls and Non-DAT Patients[a]

Diagnosis	*n*	Age (years)[b]	Plasma BChE[c]	RBC AChE[c]
Controls	9	51.2 ± 8.0	2.17 ± 0.21	1.12 ± 0.05
CVA	4	83.7 ± 4.0	1.50 ± 0.24	1.41 ± 0.27
MS	3	54.3 ± 0.3	2.03 ± 0.26	1.43 ± 0.57
DAT	14	66.8 ± 2.0	1.56 ± 0.16	1.24 ± 0.15
Presenile	9	62.1 ± 0.9	1.61 ± 0.21	1.37 ± 0.23
Senile	5	75.2 ± 2.7	1.47 ± 0.25	1.04 ± 0.14

[a]The values are given as the mean ± standard error of triplicate assays from *n* patients. CVA, cerebrovascular accident; MS, multiple sclerosis; DAT, dementia of Alzheimer type.

[b]Age at testing.

[c]μmol/hr/mg protein.

BIOL PSYCHIATRY 609
1985;20:605–610

Table 3. Levels of AChE Activity in Cerebrospinal Fluid: CSF Gradient Data[a]

Sample (ml)	Protein (mg/ml)	AChE (μmol/hr/ml CSF)
DAT		
12	0.550	0.512 ± 0.025
13	0.510	0.647 ± 0.027
22	0.592	0.631 ± 0.031
DAT		
8	0.380	0.426 ± 0.040
9–13	0.347	0.390 ± 0.025
17	0.413	0.451 ± 0.015
PTC		
1	0.350	0.395 ± 0.013
5	0.310	0.430 ± 0.022
9	0.340	0.475 ± 0.069
13	0.265	0.410 ± 0.110
16	0.280	0.475 ± 0.037

[a]Values of AChE activity are expressed as the mean ± standard error of triplicate assays on each aliquot.
PTC, pseudotumor cerebri.

investigators, we measured AChE and BChE activity in lumbar CSF from seven patients with DAT and six nondemented controls. Our data (Table 4) do not confirm the reported findings, exhibiting no significant change in AChE/BChE ratios in the CSF of DAT patients relative to the controls, although it should be mentioned that our initial measurements indicated a trend toward higher values in the DAT CSF samples that was not confirmed by statistical evaluation of a larger sample pool. In fact, it may be suggested that selective degeneration of cholinergic neurons may yield an overall increase in the ratio of true AChE to pseudo-ChE or BChE in the CSF, but the decreased ratio reported by Arendt et al. (1984) is difficult to explain in that context.

Atack et al. (1983) measured the different molecular forms of AChE in post mortem cortical tissue from DAT and age-matched normal controls. The results were very consistent in the five pairs of individuals studied, demonstrating a selective loss of the 10S, detergent-solubilized form of the enzyme. Of the total 10S enzyme activity, 73% was lost in DAT tissues, with no change in the total activity of the 4S and 16S forms. The ratio of 10S/4S forms was thus markedly decreased in DAT cortex, attaining the low levels described for cerebellum, a region poor in cholinergic synapses (Clark and Lenz 1983). We are continuing to explore the possible variations in central AChE activity in DAT by studying the distribution and ratios of the different molecular forms of AChE and BChE in different brain regions, including several cholinergic and noncholinergic regions, using post mortem tissues from DAT subjects and age-matched controls.

Table 4. Ratios of AChE/ChE Activities in Cerebrospinal Fluid of DAT and Control Patients[a]

Group	Age (*n*)	AChE[b]	BChE[b]	AChE/BChE
Control	50.8 ± 9.4 (6)	2.09 ± 0.17	0.70 ± 0.11	3.54 ± 0.60
DAT	69.3 ± 2.0 (7)	1.51 ± 0.26	0.42 ± 0.09	4.51 ± 0.51

[a]All values are expressed as the mean ± standard error of duplicate assays on (*n*) samples.
[b]μmol/hr/ml CSF.

The authors are grateful to Thomas Biagioni and Robert MacCallum for excellent technical assistance.

References

Appleyard ME, Smith AD, Wilcock GK, Esiri MM (1983): Decreased CSF acetylcholinesterase activity in Alzheimer's disease. *Lancet* ii:452.

Arendt T, Bigl V, Walther F, Sonntag M (1984): Decreased ratio of CSF acetylcholinesterase to butyrylcholinesterase activity in Alzheimer's disease. *Lancet* i:173.

Atack JR, Perry EK, Tomlinson BE, Bonham JR, Perry RH, Candy J, Blessed G, Fairbairn A (1983): Molecular forms of AChE in senile dementia of Alzheimer: Selective loss of the intermediate (10s) form. *Neurosci Lett* 40:199–204.

Clark DG, Lenz DE (1983): Characterization of the molecular forms of AChE in discrete areas of the rat brain. *Fed Proc* 42:2024a.

Deutsch SI, Mohs RC, Levy MI, Rothpearl AB, Stockton D, Horvath T, Coco A, Davis KL (1983): Acetylcholinesterase activity in CSF in schizophrenia, depression, Alzheimer's disease, and normals. *Biol Psychiatry* 18:1363–1373.

Di Chiro G, Hammock MK, Bleyer WA (1976): Spinal descent of cerebrospinal fluid in man. *Neurology* 26:1–8.

Direnfeld LK, Albert ML, Volicer L, Langlais PJ, Marquis J, Kaplan E (1984): Parkinson's disease: The possible relationship of laterality to dementia and neurochemical findings. *Arch Neurol* 41:935–941.

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 7:88–95.

Folstein M, Folstein S, McHugh PJ (1975): 'Mini-mental state,' a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12:189–198.

Lowry OH, Rosebrough NJ, Farr A, Randall RJ (1951): Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275.

Perry RH, Wilson ID, Bober MJ, Atack J, Blessed G, Tomlinson BE, Perry EK (1982): Plasma and erythrocyte acetylcholinesterase in senile dementia of Alzheimer type. *Lancet* i:174–175.

Smith RC, Ho BT, Hsu L, Vroulis G, Claghorn J, Schoolar J (1982): Cholinesterase enzymes in the blood of patients with Alzheimer's disease. *Life Sci* 30:543–546.

Soininen H, Halonen T, Reikkinen PJ (1981): Acetylcholinesterase activities in cerebrospinal fluid of patients with senile dementia of Alzheimer type. *Acta Neurol Scand* 64:217–224.

Volicer L, Direnfeld LK, Langlais PJ, Freedman M, Bird ED, Albert ML (1983): Changes of monoamine metabolism in senile dementia of Alzheimer type. *Neurosci Abs* 4:1052.

Wood PL, Etienne P, Lal S, Gauthier S, Cajal S, Nair NPV (1982): Reduced lumbar CSF somatostatin levels in Alzheimer's disease. *Life Sci* 31:2073–2079.