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A long-term follow-up study of cerebrospinal fluid acetylcholinesterase in delirium

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Abstract. Cerebrospinal fluid acetylcholinesterase (CSF AChE) was determined for elderly delirious patients during the acute stage and after a 1- and 4-year follow-up periods, and the AChE levels were compared with age-equivalent controls. The AChE levels measured during the index admission correlated with the length of life after delirium, suggesting that cholinergic dysfunction may have prognostic significance in delirious patients. Although the CSF AChE concentrations measured during the index admission were in the same range as in controls, we observed a declining trend in patients with various structural brain diseases during the follow-up period. The decreasing levels may reflect the progression of the underlying dementia in these patients.

Key words: Acetylcholinesterase – Cerebrospinal fluid – Delirium – Dementia

Introduction

Delirium is a common problem in the hospitalized elderly (Trzepacz et al. 1985; Levkoff et al. 1992). The cardinal symptoms of delirium are disorders of cognition and alteration in arousal and attention. The sleep-wake cycle is characteristically disturbed, and delusions, illusions and hallucinations are common (Lipowski 1983). Despite the varied aetiology of delirium, its clinical consequences are relatively stereotyped, which suggests that the diverse insults that cause delirium may act by a common metabolic and cellular pathway (Mesulam 1986; Gibson et al. 1991).

Altered acetylcholine activity has been suggested to underlie several neuropsychiatric disorders. Cholinergic mechanisms appear to be involved in memory, attention and arousal, which are all disturbed in delirium (Lipowski 1987; Chapouthier 1989). Cholinergic systems are exquisitely sensitive to metabolic encephalopathies and medications with anticholinergic effects, which may impair input to the cortex and hippocampus from subcortical cholinergic nuclei resulting in delirium (Itil and Fink 1966; Tune et al. 1981; Golinger et al. 1987).

Studies on cholinergic neurotransmission in living subjects have mostly relied on indirect cholinergic markers, which clearly may result controversities. One widely used cholinergic marker is cerebrospinal fluid acetylcholinesterase (CSF AChE), an enzyme which has a major role in the metabolic degradation of acetylcholine. As degeneration or dysfunction of cholinergic neurons may be reflected in the levels of CSF AChE, we decided to study whether this cholinergic marker is affected in an aetiologically heterogeneous group of delirious patients and, if so, whether this change is related to the degree of cognitive deterioration, the type of delirium or the type of basic central nervous system (CNS) disease. The results were obtained through a fourfold sampling of CSF: at entry into the study, 2 weeks after entry and 1 and 4 years after entry.

Materials and methods

Subjects

The CSF AChE was initially determined for 69 elderly patients (29 males, 40 females; mean age ± SD 74.8 ± 6.4 years, range 60–88 years) who were collected as previously described for our prospective study on delirium (Koponen et al. 1989). Patients with alcohol-related delirium were excluded, but all other delirious patients meeting the DSM-III criteria for delirium (American Psychiatric Association 1980) were consecutively included. The study design was approved by the Ethics Committee, and informed consent was obtained from all subjects or from the patient's family or legal guardian when necessary.

Evaluation of the putative CNS disease and the most important triggering factor for delirium in each patient was based on the patient's history, medical, neurological, and psychiatric examinations, and laboratory results including CT and EEG. The final diagnostic grouping was carried out according to the criteria of the DSM-III for multi-infarct dementia (MID), NINCDS-ADRDA (McKhann et al. 1984) for Alzheimer's disease (AD), and Hoehn

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Table 1. Putative aetiologies of delirium (n = 69)

	n
Stroke	14
Infection	9
Metabolic disorders	9
Epileptic fit	6
Adverse drug reactions	6
Life change in a deeply demented patient	6
Myocardial infarction of insufficiency	4
Extracranial carcinoma	4
Functional psychosis	3
Subdural haematoma	2
Intracerebral haemorrhage	2
Trauma	2
Cerebral tumour	1
Aneurysm of the basilar artery and hydrocephalus	1

and Yahr (1967) for Parkinson's disease (PD). In addition to the type of basic CNS disease, the delirious patients were divided into subgroups according to the degree of cognitive decline during acute delirium. Patients with mild cognitive decline scored from 21 to 24 on the Mini-Mental State Examination (MMSE; Folstein et al. 1975), moderate 11–20 and severe 0–10 (Duara et al. 1986). At the acute stage, delirious patients were also divided into subgroups with hyperactive, mixed or silent delirium on the basis of clinical symptoms (Lipowski 1980). The triggering factors for delirium were heterogeneous, but usually a stroke, infection, epileptic fit or metabolic encephalopathy was encountered (Table 1).

Delirium in our aetiologically heterogeneous patient group lasted 19.5 ± 15.4 days and treatment in a psychiatric hospital 30.4 ± 20.5 days. One and 4 years after the index admission the patients were again invited for a short hospital stay during which the CSF examinations and clinical ratings were repeated. Seventy-five percent (33 out of 44) of the patients still alive participated in the 1-year follow-up visit and 66% (16 out of 24) in the 4-year follow-up visit. During the follow-up period, a declining course in the cognitive functions was evident as estimated by MMSE (Table 2).

Four patients died during the index admission and 42 during the whole 4-year follow-up period, which raised the overall death rate to 66%. Autopsies, together with CNS histology, were performed in 7 cases, resulting in a histological diagnosis which was in accordance with the clinical life-time diagnosis in all cases (4 patients with vascular brain disease, 2 patients with AD, and 1 patient with no CNS disease).

Controls

The control group consisted of elderly subjects who participated in the normal aging study at Kuopio University Hospital (n = 13, 5 males, 8 females; mean age ± SD 72.4 ± 8.2 years). The sex distribution and mean age of the control group did not differ from those of the delirious patients. The controls lived independently at home, used no CNS-active drugs and had no history of neurological or psychiatric disorders, and showed no signs of dementia in the neuropsychological examination.

Lumbar punctures

The CSF sampling was performed in the morning at bed rest after an overnight fast. The first sampling was carried out on the first working day after the index admission; the second, 2 weeks after the beginning of the index admission; the third, 1 year and the fourth, 4 years after the index admission. During the 4-year follow-up visit, 5 patients were reluctant to give their consent for lumbar puncture. The first CSF aliquot (2 ml) was used for routine measurements. The next 10 ml was immediately divided into ten 1-ml portions, chilled on dry ice and frozen at −70°C until assayed.

Determination of CSF AChE

AChE activities in CSF were measured according to a modification of the colorimetric method initially developed by Ellman et al. (1961; Jolkkonen et al. 1986). The reaction mixture (3.0 ml) in the

Table 2. CSF acetylcholinesterase (AChE) concentrations in delirious patients and controls. For controls AChE 24.3 ± 6.2; n = 13. (*AChE1* CSF AChE (nmol/ml per min) (± SD) at the beginning of the index admission; *AChE2* CSF AChE 2 weeks after the beginning of the index admission; *AChE3* CSF AChE 1 year after the beginning of the index admission; *AChE4* CSF AChE 4 years after the beginning of the index admission; n number of patients; *MID* multi-infarct dementia patients; *AD* Alzheimer patients; *PD* Parkinsonian patients; *No* patients with no apparent CNS disease; *MMSE1* Mini-Mental State Examination score at the beginning of the index admission; *MMSE2* Mini-Mental State Examination score 2 weeks after the beginning of the index admission; *MMSE3* Mini-Mental State Examination score 1 year after the beginning of the index admission; *MMSE4* Mini-Mental State Examination score 4 years after the beginning of the index admission)

	Type of CNS disease				
	MID	AD	PD	No	All
AChE1	22.6 ± 8.2	21.1 ± 7.0	18.9 ± 2.1	17.9 ± 4.4**	21.3 ± 7.4
MMSE1	9.9 ± 5.4	7.6 ± 6.9	6.0 ± 6.2	12.2 ± 9.1	9.7 ± 6.6
n	39	14	3	13	69
AChE2	21.9 ± 6.6	19.2 ± 6.3	17.8 ± 3.6	19.0 ± 3.8	20.8 ± 6.2
MMSE2	14.0 ± 6.5	11.6 ± 7.9	11.0 ± 3.0	19.4 ± 9.0	13.9 ± 7.2
n	36	12	3	7	58
AChE3	19.2 ± 5.8*	17.7 ± 5.1*	18.5 ± 0.1	18.0 ± 3.1**	18.0 ± 3.1***
MMSE3	10.9 ± 6.7	3.6 ± 4.0	8.0 ± 6.1	18.3 ± 5.9	9.5 ± 7.1
n	17	8	3	5	33
AChE4	18.4 ± 5.9	17.7 ± 2.8	–	24.5 ± 0.2	19.4 ± 5.3*
MMSE4	8.1 ± 5.6	17.5 ± 2.1	–	19.4 ± 8.6	11.7 ± 9.1
n	7	2		2	11

Significance of AChE concentration differences between delirious patient subgroups and controls (two-tailed t-test):
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

total cholinesterase measurements consisted of final concentrations of 0.09 *M* phosphate buffer (final pH 7.6), 0.84 m*M* 5,5-dithiobis-(2-nitrobenzoic acid), 0.5 m*M* acetylthiocholine iodide (Sigma, USA) as a substrate and 0.5 ml of diluted CSF (1+4 with H_2O). A specific AChE inhibitor, BW284C51 (Sigma, USA) was used to inhibit true AChE and the residual activity was subtracted from the cholinesterase activity to obtain AChE activity. The mixtures were incubated at +37°C for 20 min and the yellow colour formed was read at 412 nm. Colour formation was linear for at least 30 min.

Statistical analysis

Means and standard deviations were computed for parametric variables derived from the entire delirious patient group and from the previously mentioned subgroups. Data gathered during the index admission and follow-up were compared using Student's paired sample or two-sample *t*-tests. The subgroup means were compared using one-way analysis of variance followed by Student-Newman-Keul's range statistics. Correlations between the AChE concentration, MMSE score and neuroleptic dose were calculated using Pearson's correlation technique. Analysis of covariation between the CSF AChE levels and life-span after the onset of delirium was carried out by using the Cox proportional hazards model.

Results

In the first two CSF samples taken during the index admission, the AChE levels in the whole delirious patient group were in the same range as in the controls (Table 2). The one-way procedure showed no differences between the subgroup means of delirious patients when divided according to the severity of cognitive decline, type of delirium, or CNS disease in any of the four samples.

All patients met the DSM-III criteria for delirium during the first lumbar puncture, 14 patients during the second, and none during the third and fourth CSF sampling. In patients whose delirium abated during the first 2 weeks, the paired-sample *t*-test showed no difference between the AChE levels of the first and second CSF sample (21.8 ± 8.0 versus 21.2 ± 6.2 nmol/ml per min; $P > 0.05$). In the second sample the CSF AChE concentrations did not differ between delirious and non-delirious patients (19.2 ± 6.4 versus 21.2 ± 6.2 nmol/ml per min, $P > 0.05$). The paired-sample *t*-test used to study intrasubject changes in the four samples showed no significant differences in the AChE levels between the first and second sample. There was, however, a statistically significant decline ($P < 0.05$) in the AChE levels between the second and third CSF samples in patients with AD and MID. Between the third and fourth samples there were no significant differences.

In delirious patients the correlation between CSF AChE and the MMSE score was not statistically significant in any of the four assays ($r = -0.19$, $r = -0.13$, $r = 0.09$, $r = 0.23$; all $P > 0.05$). Age had no correlation with AChE values either in the delirious patients ($r = 0.18$, $r = 0.13$, $r = -0.11$, and $r = -0.29$ respectively) or in the controls. Fifty-four of the 70 patients (77%) received neuroleptics at the beginning of the index admission, as did 23 of the 44 patients after the 1-year follow-up period (52%). After 4 years, 7 of 11 patients (64%) received neuroleptics. The respective neuroleptic doses in chlorpromazine equivalents were 143 ± 64, 133 ± 72, 115 ± 87, and 80 ± 40 mg. CSF AChE levels showed no correlation with the neuroleptic dose ($r = 0.13$, $r = 0.002$, $r = 0.15$, $r = -0.004$; all $P > 0.05$).

The annual death rates during the first, second, third and fourth follow-up year were 37%, 6%, 6%, and 17% respectively. The time between the beginning of delirium and death was 583 ± 541 days and it did not differ among the various subgroups of delirious patients divided according to the type of CNS disease. Patients who died during the follow-up period had higher CSF AChE levels at the beginning of the index admission than the rest of the patients (22.0 ± 8.0 versus 18.1 ± 5.0 nmol/ml per min; $P = 0.005$). Patients who needed prolonged institutional care in health centre wards or old people's nursing homes also had higher AChE levels in the first sampling (21.0 ± 5.9 versus 17.8 ± 4.5 nmol/l per min; $P = 0.05$). The proportional hazards model identified an association between the CSF AChE levels taken at the beginning of the index admission and 2 weeks after the beginning of the index admission and with the life-span after delirium ($z = 2.39$, $P = 0.019$; and $z = 2.55$, $P = 0.018$ respectively).

Discussion

Age-related decline in cholinergic neurotransmission and concurrent medical illnesses requiring the use of multiple medications are widely recognized risk factors for delirium in the elderly (Lipowski 1980). Thus dysfunction in cholinergic neurotransmission has been suggested to be important in various forms of delirium (Gibson et al. 1991). In this study CSF AChE was chosen as a potential marker which might reflect central cholinergic cell loss or dysfunction.

In our series, however, the observed CSF AChE concentrations during the index admission in the whole delirious patient group were in the same range as in the controls. The caudate nucleus and substantia nigra near the lateral ventricles make the most important contribution to the total CSF AChE (Hollander et al. 1986). Since these brain regions are not consistently affected in delirium and various dementias, this could account for the fact that the AChE levels during the index admission did not differ from those of the controls. During the follow-up period significant differences between delirious patients and controls evolved.

Differences between patients with MID and AD or PD and controls were not significant in the first two samples. These results are in accordance with previous observations in the sense that cognitive deterioration in these patients may occur without an involvement of AChE in the lumbar CSF (White et al. 1977; Perry et al. 1978; Jolkkonen et al. 1986; Nakano et al. 1986; Appleyard et al. 1987; Elble et al. 1987; Riekkinen et al. 1987; Sirviö et al. 1987). In previous follow-up studies, the AChE levels in AD patients were quite stable over a span of 12 months (Elble et al. 1987; Atack et al. 1988). In our patients, however, the progression of dementia in patients with AD and MID was associated with declining lumbar AChE levels, which may be related to an increasing cholinergic dysfunction in these patients. In patients with no overt CNS disease, the AChE levels were in the same range as in patients with

AD, PD or MID. However, in the first and third samples their CSF AChE levels were lower than those of the control group, suggesting that a subtle cholinergic dysfunction may also increase susceptibility to delirium in patients with no apparent CNS disease (Drachman and Leavitt 1974; Dubois et al. 1987).

Central acetylcholine turnover and CSF AChE activity are affected by certain drugs, e.g. haloperidol and chlorpromazine (Greenfield et al. 1979). However, CSF AChE concentrations have not correlated with neuroleptic dose in previous clinical studies (Soininen et al. 1981, 1984; Appleyard et al. 1987; Sirviö et al. 1987; Huff et al. 1988). This was also the case in our series. The lack of correlation between neuroleptic dose and AChE levels and the lack of differences between medicated and unmedicated patients suggests that neuroleptic treatment has not affected our results, although it cannot be excluded with certainty.

In some studies, CSF cholinesterase levels correlated with the severity of dementia in AD patients (Soininen et al. 1981, 1984). However, in other studies no such correlations have been found, e.g. in PD (Sirviö et al. 1987) or AD (Tune et al. 1985; Atack et al. 1988). In our patients, correlations between CSF AChE and MMSE scores were not significant, which suggests inconsistencies in the relationship between CSF AChE and cognition.

In delirious patients we observed higher AChE levels in those patients who needed prolonged institutional care or who deceased during the follow-up period. This may reflect an excessive release of this enzyme from degenerating cholinergic neurons in the brain. On the other hand, AChE containing neurons in the striatum, caudate nucleus, cerebellum and spinal cord probably also contributes to lumbar CSF AChE activity, which may mask reduced cortical AChE secretion, e.g. in patients with hyperkinetic or mixed delirium (Atack et al. 1986; Weston and Greenfield 1986). These subtypes characterized most of our delirious patients, and they also had the highest mortality rate during the follow-up period.

The systemic diseases triggering delirium appear to alter brain function by interfering with metabolism in wide areas of the brain. The cholinergic system appears to be particularly vulnerable to metabolic insults, and the anatomical distribution of cholinergic neurons is such that changes in the cholinergic system can profoundly influence overall brain function (Gibson et al. 1991). Although degeneration of central cholinergic neurons might be expected to result in decreased AChE levels, we did not find significant reductions of CSF AChE during acute delirium in this study. The observed association between the AChE levels during acute delirium and length of life after delirium suggests, however, that involvement of cholinergic neurons may have prognostic implications in delirium.

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