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CHOLINERGIC ENZYMATIC ACTIVITY OF CEREBROSPINAL FLUID OF PATIENTS WITH VARIOUS NEUROLOGIC DISEASES*

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SUMMARY

The cerebrospinal fluid (CSF) of normal humans contains both true and pseudo-cholinesterases and choline acetyl transferase (choline acetylase) detectable by radio-chemical assay. Elevations of total cholinesterase, pseudocholinesterase, and true cholinesterase occur in brain tumors, meningitis, Guillain-Barre disease, hydrocephalus, and brain abscess. However, the non-specificity and inconstancy of CSF cholinesterase changes in neurologic diseases limit its clinical usefulness.

Choline acetylase levels in CSF are normally 0.187 ± 0.073 nmoles ACh/ml CSF/min. One patient with meningioma had a level of 0.680. Unlike cholinesterase, choline acetylase levels in blood are lower than in CSF. Hence, the origin of elevated CSF choline acetylase activity is probably in the nervous system.

INTRODUCTION

Measurement of serum enzymes provides a useful diagnostic clue in patients with hepatitis, myocardial infarction, muscular dystrophy, and other somatic diseases. By analogy, one might expect enzymes released into the cerebrospinal fluid (CSF) from the brain to help in the diagnosis of neurologic diseases. However, assay of spinal fluid enzymes has not gained widespread clinical acceptance. Earlier studies of CSF enzymes have been criticized because the origin of these enzymes is not clear. We have reexamined CSF cholinesterases, reviewed previous reported changes in CSF cholinesterase in diseases of the nervous system, and sought further correlations between cholinesterases and neurologic disease. In addition, we applied a similar approach to the enzyme choline acetylase (choline acetyltransferase) which, to our knowledge, has not previously been measured in human CSF.

METHODS

Cholinesterase

Acetyl- and butyrylcholinesterase were assayed using the radiochemical technique of Siakotos *et al.*[1]. Activity was measured as the rate of hydrolysis of various ^{14}C-labelled choline ester substrates. After 10 min incubation with CSF at $37°$, addition of Amberlite CG-120 cation-exchange resin was used to bind unhydrolyzed substrate. Initial substrate concentration was $3 \cdot 10^{-3}$ M and contained enough radioactivity to yield 20 000 disintegrations per minute. Acetylcholinesterase ("total"

TABLE I

CHOLINESTERASE ACTIVITY IN NEUROLOGIC DISEASES

| Disease	Number of patients	Mean CSF protein in mg %	Cholinesterase activity in nmoles substrate/ml CSF/min Mean and standard deviation for each substrate		
			Acetylcholine	*Methacholine*	*Butyrylcholine*
'Normal' inpatients	7	37	17.5 ± 4.1	1.2 ± 0.9	11.3 ± 2.9
Hydrocephalus	4	84	21.6 ± 7.2	1.2 ± 0.6	40.1 ± 26.2
Multiple sclerosis	6	43	19.9 ± 5.8	2.4 ± 2.1	15.0 ± 5.8
CNS leukemia and lymphoma	8	102	20.7 ± 10.6	0.1 ± 0.2	21.5 ± 16.5
Guillain-Barré	2	246	28.5 ± 3.3	0.6 ± 0.7	62.5 ± 5.0
Meningitis	3	261	22.7 ± 3.2	0.9 ± 1.3	54.0 ± 42.1
Senile dementia	7	36	22.9 ± 11.0	2.1 ± 1.7	10.1 ± 4.7
Leber's optic atrophy	1	16	21.7	3.7	6.6
Trigeminal neuralgia	1	23	10.3	0.1	24.9
B-12 neuropathy	1	42	17.4	0.9	19.7
Pseudotumor cerebri	1	30	22.4	0.7	6.9
Parkinson's disease	1	31	20.0	0.6	11.9
Ependymoma	1	29	3.5	1.5	10.3
Acoustic neuroma	1	16	21.3	0.7	12.0
Subacute sclerosing panencephalitis	1	–	32.7	2.2	13.5
Syphilis	1	82	20.7	2.2	11.2
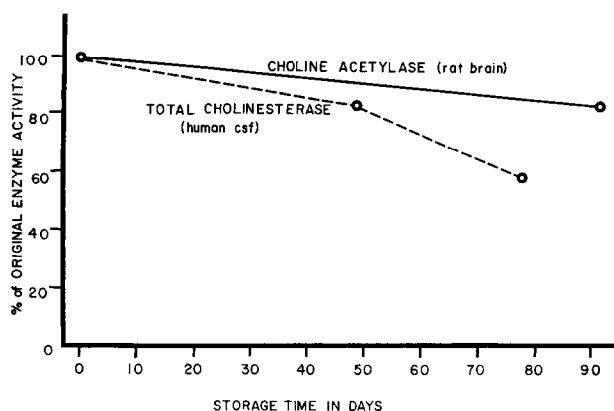
Fig. 1. Loss of enzyme activity on storage at $-25°$ C. The activity of both total cholinesterase and choline acetylase on storage is shown. The slight loss of enzyme activity during the experiment was not significant (see details under Methods).

cholinesterase) activity was measured using [1-14C]acetyl choline substrate. Metha-choline hydrolysis ("true" cholinesterase) was measured using [1-14Cacetyl] beta methacholine plus $5 \cdot 10^{-6}$ M tetra-isopropylpyraphosphoramide (iso-OMPA), a butyrylcholinesterase inhibitor. Since iso-OMPA also inhibits acetylcholinesterase somewhat, our values are lower than previously reported activities (see Table I). Butyrylcholinesterase ("pseudocholinesterase") activity was determined with [1-14C]-butyrylcholine substrate plus 10^{-7} M B.W. 28451, a true cholinesterase inhibitor.

Samples of CSF were assayed in batches. Mean storage time of samples was 15 days, a maximum storage time was 33 days in one instance. Fig. 1 shows that a slight loss of cholinesterase activity did occur in storage at $-25°$ C. However, mean storage time of the samples in the various diagnostic categories did not significantly differ.

Choline acetylase

Choline acetylase was assayed by a radiochemical method based on the work of McCaman and Hunt[2] and Schrier and Shuster[3]. In this assay, choline acetylase forms acetylcholine from excess choline (10^{-2} M) and 14C-labelled acetyl-coenzyme A ($6.25 \cdot 10^{-4}$ M, 20 000 cpm). The labeled acetylcholine formed was separated on prewashed 8 cm Dowex 1-X8 Cl (200–400 mesh) columns. The purified acetylcholine radioactivity was determined on a scintillation counter.

Since the CSF contains only small amounts of choline acetylase activity, sam-ples were concentrated 10 times by lyophilization before assay. Aliquots of pooled CSF from 25 patients were run with each batch of choline acetylase assays to control the uniformity of the procedure.

Loss of rat brain homogenate choline acetylase activity with storage is also shown in Fig. 1. Mean storage time of samples was 65 days, ranging from 14 to 99 days. Mean storage times of samples in the various diagnostic categories did not significantly differ.

Protein

Cerebrospinal fluid protein was determined by the method of Lowry *et al.*[4]

Cerebrospinal fluid

Cerebrospinal fluid was obtained from three sources:(a) patients with neurologic diseases had lumbar punctures performed by the clinical staff of the Neurology, Neurosurgery and Pediatrics Departments. The fluid was kept refrigerated for a maximum of 48 h at $4°$ before freezer storage at $-25°$. (b) CSF was obtained at pneu-moencephalography to determine if any relationship existed between the amount of fluid withdrawn, the protein concentration, and enzyme activity. With the patient sitting, serial samples of fluid were withdrawn as increasing amounts of air were injected. CSF not needed for other clinical measurements was saved for enzyme ana-lysis. (c) Five samples of 2 ml CSF from normal volunteers was obtained through the courtesy of Professor W. W. Tourtelotte of the Neurology Department. Samples of 20 ml CSF were obtained by lumbar puncture at the rate of 1 ml/min. The samples were spun down and the supernatant frozen at $-80°$ in 1 ml aliquots. The samples had been frozen 8 months at the time of assay.

Patients were assigned to diagnostic categories according to information obtained from their hospital records. The following criteria were used:

(1) Normal controls: (a) CSF from 5 normal college student volunteers courtesy of W.W.T. (see above). The students had a normal review of systems, no history of metabolic, neurologic, or other disease, and no history of drug abuse. They had normal neurologic examinations, CSF cell counts, VDRL's, and CSF protein levels. This group was used for choline acetylase assays only. (b) In patients who had had extensive clinical and laboratory workups, but who had no pathologic findings, or at most a diagnosis of "neurosis."

(2) Hydrocephalus: Bulging fontanelle, head circumference two standard deviations or more above normal for age, signs of increased intracranial pressure, on ventriculography no sign of a mass or lesion.

(3) Multiple sclerosis: Evidence of multiple central nervous system (CNS) lesions both spatially and temporally, usually including retrobulbar neuritis.

(4) Senile dementia: Signs of disorientation and loss of recent memory in a person over 50 years old. On pneumoencephalography, cortical atrophy and/or ventricular dilatation were present but no evidence of a mass or lesion.

(5) Leukemia or lymphoma involving CNS. Histologic evidence of leukemia or lymphoma. History of seizures beginning after onset of disease, with malignant cells in the CSF.

(6) Meningitis: Fever, stiff neck, CSF leukocytosis plus appropriate bacterial cultures of CSF.

(7) Seizure disorder: History typical of psychomotor or grand mal epilepsy, and EEG evidence of seizure focus. No mass or lesion was demonstrable.

(8) Guillain-Barré: Largely reversible ascending paralysis associated with a previous upper respiratory infection and large CSF protein elevation.

(9) Tumor: Both primary brain and metastatic tumors provided by histologic examination.

(10) Herniated disk: Myelography and operative confirmation.

The other diagnoses were made by the clinical staff at University Hospital, but full diagnostic details will not be given.

RESULTS

Cholinesterase

Surprisingly, cholinesterase activity in the CSF did not vary with CSF total protein nor with the amount of CSF withdrawn (up to 30 ml) in a patient with cerebellar atrophy. We obtained similar results in a patient with multiple sclerosis, one with senile dementia, and one who developed thalamic pain two years after a stroke. In a fifth patient who had psychomotor epilepsy, the graph showed a rough correlation between butyrylcholinesterase activity and total protein, but a coefficient of correlation was not significant. Hence, we express CSF cholinesterase activity per unit volume of CSF.

Table I shows cholinesterase activity in the CSF of patients with neurologic disease. The following results differ significantly from normal as measured by the

student's two tailed t-test: low true cholinesterase activity in leukemia-lymphoma; elevated protein, total cholinesterase, and pseudocholinesterase activity in Guillain-Barré disease; and elevated pseudocholinesterase activity in meningitis. Total protein in meningitis, and pseudocholinesterase activity in hydrocephalus, had t-values such that $P = 0.06$, and these values would probably be significantly elevated in a larger series.
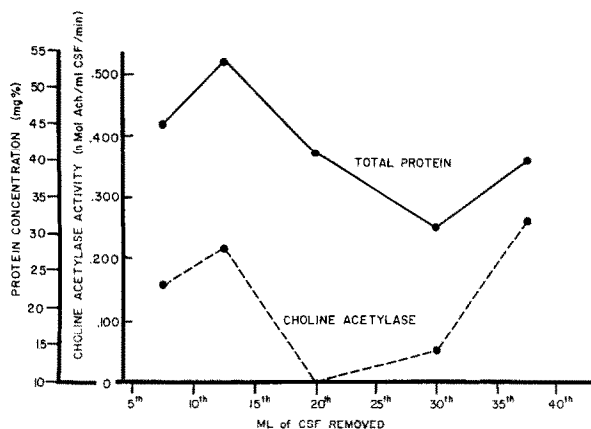
Choline acetylase

Fig. 2. Change in choline acetylase activity and protein concentration with removal of spinal fluid. Choline acetylase activity fluctuated as spinal fluid was withdrawn and appeared to vary with protein concentration in CSF. Hence, choline acetylase activity was expressed both per ml CSF and per mg CSF protein (see Discussion).

TABLE II

CSF CHOLINE ACETYLASE ACTIVITY IN NEUROLOGIC DISEASES

Disease	Number of patients	Mean CSF protein in mg %	Choline acetylase activity	
			nmoles/ml CSF/ min mean + S.D.	nmoles/mg protein/ min mean ± S.D.
Normal college students	5	55	0.187 ± 0.073	0.348 ± 0.127
'Normal' inpatients	4	52	0.145 ± 0.120	0.248 ± 0.237
Senile dementia	4	49	0.160 ± 0.018	0.328 ± 0.032
Brain tumor	5	125	0.252 ± 0.115	0.430 ± 0.694
Meningioma	1	42	0.680	1.62
Aseptic meningitis and encephalitis	3	108	0.093 ± 0.092	0.125 ± 0.147
Multiple sclerosis	6	63	0.143 ± 0.092	0.255 ± 0.160
CNS leukemia/lymphoma	7	86	0.150 ± 0.098	0.287 ± 0.333
Leukemia	1	27	0.265	0.984
Herniated disk	3	64	0.097 ± 0.052	0.145 ± 0.067
Hydrocephalus	3	102	0.027 ± 0.043	0.047
Seizure disorder	5	46	0.208 ± 0.072	0.446 ± 0.097
Pseudotumor cerebri	2	56	0.122	0.218
Parkinson's disease	1	64	0.158	0.245
Cerebrovascular accident	1	92	0	0
Leber's optic atrophy	1	22	0.172	0.780
Amyotrophic lateral sclerosis	1	75	0.052	0.068
B-12 neuropathy	1	65	0.195	0.300
Guillain-Barré	1	160	0.244	0.152

Fig. 2 shows graphically the relationships among the amount of CSF withdrawn, the choline acetylase activity and the total protein concentration in CSF in a patient with senile dementia. Choline acetylase activity which is extremely low in CSF seems to vary with total protein concentration. In another patient who had multiple sclerosis, the choline acetylase activity appeared to vary with the protein, too. The coefficient of correlation between protein and choline acetylase activity was not significant using data from only two patients, however. Hence, we have expressed choline acetylase activity both per ml of CSF and per mg CSF protein.

Table II shows CSF choline acetylase activity in neurologic diseases. The normal level varies from o to about 0.35 nmoles per ml CSF per min or from o to about 0.60 nmoles per mg CSF protein per min. None of the diagnostic groups differed significantly from normal. However, a patient with a meningioma had levels over 4 S.D. above the normal mean whether expressed per ml of CSF or per mg CSF protein. A sample from one of the patients with CNS leukemia and one of the samples from the patient with Leber's hereditary optic atrophy were well above the normal range expressed per mg CSF protein.

DISCUSSION

To make sense of the measurement of cholinesterase in CSF, several questions must be answered: Where does the cholinesterase come from? Does the concentration of cholinesterase vary with the source of the sample, *i.e.*, lumbar, cisterna magna or ventricular? Does the protein concentration or the number of cells present affect the activity? In general, neurons and erythrocytes contain most of the true cholinesterase; pseudocholinesterase is found predominantly in glial cells and in serum[4-8]. Studies on pathological specimens reveal decreased cholinesterase activity in multiple sclerotic plaques[9] and increased pseudocholinesterase activity in brain tumor tissue[10]. Cholinesterase in CSF might originate from any of these sites.

Concerning possible sampling variations in CSF cholinesterase activity, our data show remarkably constant activity, whether the first or the 35th ml of CSF withdrawn is assayed. Surprisingly, our data show that CSF cholinesterase activity and total protein vary independently. Colling and Rossiter[11] reported a significant correlation between pseudocholinesterase activity and total CSF protein. Jefferson[12] and Tower and MacEachern[13] reported correlation between pseudocholinesterase and total protein only when total protein levels are greater than 50 mg%. To explain the apparent contradiction, note that cholinesterase has one of the highest substrate turnover numbers of any enzyme. Thus, cholinesterase activity could change greatly with little increase in total protein. Furthermore, Colling and Rossiter's report included many cases of meningitis, where the blood-cerebrospinal fluid barrier is known to have increased permeability to plasma proteins. Influx of plasma protein, which contains high pseudocholinesterase activity, would account for a simultaneous rise and a significant correlation between the two.

The number of cells in the CSF has little effect on cholinesterase activity. Leukocytes have insignificant cholinesterase levels[14,15]. Birkhauser[16], Collin and Rossiter[11], and Tower and MacEachern[13] observed no correlation between cholinesterase activity and CSF leukocyte counts. Similarly, Tower and MacEachern[13] observed no relationship between CSF erythrocyte counts and cholinesterase activity. A theoretical

computation based on their data shows that 2000 erythrocytes/mm³ would raise the normal rate of acetylcholine hydrolysis by only 10%.

   Changes in cholinesterase activity in neurological diseases reported in this paper and by previous investigators are summarized in Table III. The table is organized according to the possible origins of CSF cholinesterase discussed earlier. Thus, in diseases where the blood-cerebrospinal fluid barrier is abnormally permeable, plasma cholinesterase, *i.e.*, pseudocholinesterase, may leak into the cerebrospinal fluid. This may explain the increased pseudocholinesterase activity described in meningitis, Guillain-Barré disease, and hydrocephalus. Similarly, some brain and spinal cord tumors contain high levels of cholinesterase, and elevations of CSF total cholinesterase have been observed in patients with brain tumor. Destruction of brain parenchyma would be expected to release brain cholinesterase into the CSF, and this may be the mechanism of elevated cholinesterase levels found in the CSF of some patients with brain abscess. Finally, low levels of cholinesterase have been reported in multiple sclerotic plaques, and the most extensive investigation of CSF cholinesterase in multiple sclerosis patients showed low levels of true cholinesterase activity[19]. The reports of Jefferson[12], Webster and MacKenzie[20] and our own data show no difference between normal and multiple sclerosis in CSF pseudocholinesterase activity. Our data and that of Jefferson[12] do not confirm the findings of Plum and Fog[19] of decreased true cholinesterase activity in multiple sclerosis, but we tested far fewer patients than Plum and Fog.

TABLE III

CSF CHOLINESTERASE CHANGES IN NEUROLOGIC DISEASE,
ARRANGED BY PROBABLE ORIGIN OF CHOLINESTERASE

A. Plasma-Abnormally permeable blood-CSF barrier
   (1) Meningitis – elevation of pseudocholinesterase[11,16,17]   and our results.
   (2) Guillain-Barré – elevation of pseudocholinesterase[18] and our results
   (3) Hydrocephalus – elevated pseudocholinesterase – our results.

B. Brain tumor – elevation of total cholinesterase activity[17]

C. Destruction of brain parenchyma – abscess – elevation of total cholinesterase activity [17]

D. Multiple sclerotic plaques – decreased true cholinesterase [19]

E. Other – myasthenia gravis – elevated true cholinesterase[18]

   Despite a theoretical framework to account for changes in CSF cholinesterase, the practical significance of the changes is minimal. Cholinesterase elevations in disease states are either non-specific or do not occur in enough of the patients with the disease to be of help in diagnosis. In a large number of neurological and psychiatric diseases, cholinesterase activity is normal[11,17,18,21-25].

   Choline acetylase is present in cerebrospinal fluid, but in very much smaller quantity than cholinesterase. The ratio of normal mean enzyme levels in CSF is about 1 part choline acetylase to 100 parts cholinesterase. When levels are expressed as enzyme activity per mg protein, the ratios of brain enzyme to CSF enzyme are surprisingly similar for cholinesterase and choline acetylase. The average cortical total cholinesterase is 152, and the caudate nucleus total cholinesterase 7,602 μl CO_2/g wet brain/10 min, as reported by Okinaka *et al.*[5]. Bull *et al.*[26] reported choline acetylase activity of 190 in gray matter of cortical area 4, and 3790 μg ACh/g fresh brain/h in

caudate nucleus. Using our data of 46.6 nmoles ACh/mg CSF protein/min for total cholinesterase, 0.348 nmoles ACh/mg CSF protein/min for choline acetylase activity in normal CSF, and 100 mg protein per gram brain tissue, we obtained the following ratios after conversion of units: Choline acetylase/mg protein: CSF/cortex = 1.6, CSF/caudate = 0.08, caudate/cortex = 20. Total cholinesterase/mg protein: CSF/cortex = 6.7, CSF/caudate = 0.14, caudate/cortex = 50. That these ratios are so similar suggests that both enzymes normally reach the spinal fluid from the same source, probably brain tissue. However, unlike cholinesterase, choline acetylase is not found in high concentrations in serum and erythrocytes. Using the same assay method as for CSF, we found that serum choline acetylase activity is about the same or less than that in CSF, while erythrocyte activity is about twice as high as CSF activity. Hence, blood is not an important source of elevated CSF choline acetylase activity.

The results in Table II demonstrate that choline acetylase is normally present in CSF and that it can be elevated in patients with neurologic disease. Its clinical usefulness, if any, awaits the findings of elevated CSF choline acetylase in a neurologic disease not better diagnosed by other methods. Neurologic diseases involving active destruction of brain tissue might be expected to show higher than normal CSF choline acetylase activity.

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