Cerebrospinal fluid proteins and free amino acids in patients with solvent induced chronic toxic encephalopathy and healthy controls

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Abstract

The concentrations of protein, albumin, IgG, and free amino acids in the cerebrospinal fluid of 16 patients with chronic toxic encephalopathy due to organic solvents were measured. The patient group consisted of all patients with this diagnosis in a neurological department in 1985. The diagnosis was based on neuroaesthetic symptoms, pathological psychometric performance, and verified exposure to neurotoxic organic solvents. A control group of 16 patients with myalgias or backache, or both, and no signs of disease was used for comparison. The purpose was to study possible changes in the cerebrospinal fluid that might contribute to understanding the aetiology of solvent induced chronic toxic encephalopathy. A rise in protein, albumin, and IgG was found in the patient group compared with the control group, as well as reduced concentrations of phosphoethanolamine, taurine, homocarnosine, ethanolamine, alpha-aminobutyric acid, and leucine. Using a stepwise multiple regression analysis, taurine was negatively correlated to exposure to solvents. These findings may indicate membrane alterations in the central nervous system related to exposure to organic solvents.

Case reports^{1,2} and epidemiological studies³⁻⁵ of workers occupationally exposed to organic solvents suggest the existence of a chronic toxic encephalopathy. This diagnosis has been debated for some time but the syndrome is generally accepted by occupational toxicologists. The World Health Organisation and a working group in the United

States have suggested classification systems for the syndrome.^{6,7}

An increase of proteins and albumin ratio has been found in the cerebrospinal fluid of patients exposed to solvents.⁸ These findings may be related to aetiological mechanisms of the syndrome and the present study was made to examine this further. Amino acids and proteins in the cerebrospinal fluid were studied. Amino acids are precursors of several neurotransmitters and some of them may themselves act as neurotransmitters or modulators of neural activity.⁹ Some amino acids have been proposed as neuromodulators with behavioural relevance.^{9,10} This may be of importance concerning chronic toxic encephalopathy as behavioural changes are seen in these patients. No studies of amino acid concentrations in the cerebrospinal fluid of patients with chronic toxic encephalopathy due to organic solvents have previously been performed.

Material and methods

PATIENTS

At our department of neurology in 1985 19 patients were diagnosed as having chronic toxic encephalopathy. The diagnosis was made after an interview with the patient, a general and a neurological examination, neuromyographic measurements, electroencephalography, registration of visual and auditory evoked potentials, a computed cerebral tomogram, and an examination of the cerebrospinal fluid. Furthermore, the patients performed neuropsychological tests including tests of psychometric intelligence and memory (WAIS), tests of adaptive sensory and motor functions (Halstead-Reitan battery), and a personality test (MMPI). Age and educational level were considered when evaluating the results. In addition, the following blood analyses were performed; haematological examination, serum electrolyte study, liver function tests, serological test for syphilis, serum vitamin B12 level, thyroid function studies, serum triglyceride level, serum cholesterol level, serum immunoglobulin level, fasting serum glucose level, and a glucose tolerance test. Urine was examined for the presence of glucose, protein, and blood, and a microscopic study was performed. The diagnosis was based on the presence of neuroaesthetic

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Table 1 Occupations and organic solvent exposure in 16 patients with chronic toxic encephalopathy

Occupation	No of patients	Exposure
Painter/spraypainter	6	Mixtures of hydrocarbons, mostly xylenes
Worker in paint industry	5	Mixtures of hydrocarbons, mostly xylenes, n-hexane
Worker in plastic industry	2	Mixtures of hydrocarbons, mostly styrene, acetone
Worker in printing industry	1	Mixtures of hydrocarbons, mostly toluene, xylenes, trichloroethylene
Worker in mechanical industry	1	Mixtures of hydrocarbons, mostly trichloroethylene
Able seaman on chemical tanker	1	Mixtures of hydrocarbons, mostly toluene, hexanes, styrene, xylenes

symptoms and abnormal performance in psychometric tests preceded by a substantial exposure to neurotoxic organic solvents (table 1). In addition, other diseases were excluded as a primary cause to the symptoms and signs of disease. All patients had central nervous symptoms such as memory impairment, concentration difficulties, vertigo, headache, excessive fatigue, depression, and anxiety. Four had symptoms relating to the peripheral nervous system; muscular weakness or paresthesias or both (table 2). All patients had a clearly reduced functional intellectual capacity and major deficits in cognitive functioning such as memory and concentration. Some also had impaired sensory motor capacity. Five had diffuse cerebral atrophy and two cerebellar atrophy by a subjective evaluation of an experienced neuroradiologist. Four had an abnormal EEG with increased theta activity over the anterior parts of the

brain or over the lateral parts of one or both sides; polyneuropathy was found in four. According to the classification suggested in the United States they had a type 2b syndrome.⁷

One patient refused to undergo a lumbar puncture and two of the specimens of cerebrospinal fluid were lost before the analyses could be performed, leaving 16 samples available for analysis. The 16 patients were men aged from 20 to 67. Their mean exposure to organic solvents was 24 years, ranging from eight to 52 years.

CONTROL GROUP

The control group consisted of 16 patients examined in our department who had been referred because of myalgia or backache, or both. They had a normal general and neurological status and further investigations by blood samples, EEG, and computed tomograms of cerebrum and spine did not show any indication of lesion of the nervous system. The 10 women and six men aged between 21 and 68 had had no appreciable exposure to organic solvents. They had a similar socioeconomic and educational level as the patient group.

CEREBROSPINAL FLUID ANALYSIS

The cerebrospinal fluid (10 ml) was obtained by a standardised procedure. A lumbar puncture was performed with the patient in supine position, before breakfast, after at least eight hours resting. It was stored at -20°C until the analysis was made. Fluid for the analyses was taken from the pooled cerebrospinal fluid volume of 10 ml. None of the samples was contaminated by blood. No abnormalities were found in the cerebrospinal fluid as judged by cell count and electrophoresis in either the exposed or the unexposed group. The mean cell value was $2.2 \times 10^6/\text{l}$ in the patients (range 0.8–5.3) and $1.9 \times 10^6/\text{l}$ in the control group (range 0.4–5.0).

Table 2 Age, symptoms, and pathological findings in 16 patients with chronic toxic encephalopathy due to organic solvents

Patient No	Age	Symptoms		Pathological findings by examination				
		CNS	PNS	Neurological examination	Neurography	EEG	Cerebral CT	Psychological testing
1	67	+	–	+	–	+	–	+
2	66	+	+	+	–	–	+	+
3	65	+	–	–	–	+	+	+
4	64	+	–	+	–	+	+	+
5	63	+	+	+	+	–	+	+
6	63	+	–	+	–	–	–	+
7	58	+	–	+	–	+	–	+
8	55	+	–	–	–	–	–	+
9	50	+	+	+	+	–	+	+
10	48	+	–	–	–	–	–	+
11	45	+	–	–	–	–	–	+
12	37	+	+	+	+	–	+	+
13	35	+	–	–	–	–	–	+
14	35	+	–	–	–	–	–	+
15	33	+	–	+	–	–	–	+
16	29	+	–	–	–	–	–	+

Proteins were separated by agarose gel electrophoresis. Protein concentrations were determined by the folin phenol reagent method.¹¹ Concentrations of albumin and immunoglobulin G were measured by a laser nephelometric analysis. Amino acid concentrations were quantitated by high performance liquid chromatography after precolumn derivatisation with o-phthalaldehyde, as described previously¹² (and BA Engelsens *et al*, unpublished data). The concentrations were expressed in $\mu\text{mol/l}$.

STATISTICAL ANALYSES

The differences between the two groups regarding concentrations of total protein, albumin, IgG, and amino acids in the cerebrospinal fluid were found by using the Student's *t* test (two tailed).

The correlations between the measured variables in cerebrospinal fluid, age, and an exposure index were estimated by the use of multiple regression analyses. Age was adjusted by including this variable in all steps of the analyses. The exposure index was estimated for each patient by multiplying the number of years of exposure to solvents with the mean daily exposure time and a factor that rated the degree of exposure from one to four.

Results

The concentrations of total protein, albumin, and IgG were significantly higher in the patient group compared with the control group (table 3).

Table 3 Total protein (g/l), albumin (g/l), IgG (g/l), and amino acid ($\mu\text{mol/l}$) concentrations in the cerebrospinal fluid from patients with chronic toxic encephalopathy compared with a reference group

	Reference group (n = 16)		Exposed group (n = 16)		p Value*
	Mean	SD	Mean	SD	
Total protein	0.347	0.11	0.480	0.17	0.01
Albumin	0.201	0.06	0.301	0.13	0.0057
IgG	0.026	0.09	0.053	0.05	0.0248
Phosphoserine	0.3	0.2	0.3	0.2	NS
Aspartic acid	1.8	0.9	1.6	0.7	NS
Asparagine	4.4	2.6	4.2	1.8	NS
Glutamic acid	2.3	1.5	2.3	1.3	NS
Histidine	6.2	3.6	4.1	2.5	NS
Serine	22.9	6.7	21.1	11.8	NS
Glutamine	607.7	181.5	519.2	200.1	NS
Citrulline	1.8	0.9	1.5	0.8	NS
Arginine	24.8	8.6	18.8	9.1	NS
Threonine/glycine	30.0	9.4	29.8	16.1	NS
Phosphoethanolamine	2.3	0.7	1.5	0.7	0.0058
Taurine	8.8	3.1	5.1	2.0	0.0002
Thyrosine/ β -alanine	7.8	4.2	6.8	3.2	NS
Homocarnosine	0.8	0.4	0.4	0.2	0.0007
Alanine	27.1	9.5	26.9	17.1	NS
γ -Amino butyric acid	0.3	0.2	0.2	0.1	NS
Ethanolamine	22.5	7.8	12.9	5.5	0.0003
α -Amino butyric acid	3.6	1.9	2.4	1.3	0.0428
Tryptophane	1.8	1.1	1.4	0.5	NS
Methionine	3.2	1.4	2.5	1.3	NS
Valine	15.0	5.8	14.8	7.9	NS
Phenylalanine	8.2	3.1	6.4	3.2	NS
Isoleucine	4.9	2.0	3.8	1.6	NS
Leucine	14.7	6.1	9.8	3.7	0.0088
Lysine	39.6	21.4	49.4	27.9	NS

*Two tailed *t* test.

The IgG/total protein ratio was higher in the patient group (0.108) than in the control group (0.074). The concentrations of phosphoethanolamine, taurine, homocarnosine, ethanolamine, α -aminobutyric acid, and leucine were significantly lower in the patient group than in the control group (table 3).

In multiple regression analyses of both the patient and reference groups taurine showed a significant negative correlation with increasing solvent exposure ($r = -0.365$, $p = 0.045$). Most of the other amino acids were negatively correlated with increasing exposure but the correlations were statistically insignificant. Multiple regression analyses were performed analysing the relations to both exposure to organic solvents and age. Age did not interfere with the reported correlations to exposure. Neither age nor exposure showed any correlation to total protein, albumin, or IgG. Multiple regression analyses of the patient group only gave almost identical results as when the two groups were analysed together.

Discussion

Raised cerebrospinal fluid concentrations of total protein, albumin, and IgG and reduced concentrations of some free amino acids were found in the patients with chronic toxic encephalopathy compared with the control group.

The increased concentrations of protein and

albumin may be due to a protein leak through the blood brain barrier or to reduced cerebrospinal fluid drainage.

The reductions of free amino acid concentrations are difficult to interpret as the functions of the amino acids are still a matter of controversy. Changes in cerebrospinal fluid free amino acid concentrations have been found in some neurological and metabolic disorders¹³ but the clinical relevance of these findings is not known. An additional problem in interpreting the present findings is the methodological differences between the studies. Several studies have been performed to establish normal values of free amino acids in cerebrospinal fluid^{14,15} but they are difficult to compare and to use in clinical practice. Because of these differences, a control group was used in the present study.

In our studies the concentration of taurine had a significant negative correlation with exposure to organic solvents. The other amino acid concentrations that were significantly lower in the exposed group compared with the unexposed group showed a similar correlation with exposure but the correlations were not significant. This suggests that the finding is real and not an accidental statistical occurrence due to the high number of analyses performed.

The neurobiological functions of taurine are not yet unequivocally established. Taurine may be a neurotransmitter or function as a neuromodulator.^{9,13} It has membrane stabilising and inhibitory effects and may be linked to the calcium flow in excitable tissue.^{9,16,17} Taurine may protect cell membranes and cells by attenuating toxic compounds that generate oxidants.¹⁸ Furthermore, a reduced cerebrospinal fluid taurine concentration has been reported in patients with hereditary mental depression and parkinsonism¹⁰ and in patients with a reduced level of consciousness.¹⁹

Acute and chronic exposure to organic solvents may alter membrane fluidity in the central nervous system.²⁰ Similar effects occur when the concentration of taurine is reduced.¹⁶⁻¹⁸ The mechanisms regarding these effects and a possible interaction between taurine and organic solvents are unknown.

Interrelations between levels of extracellular taurine and phosphoethanolamine have been seen in an experimental study and it is suggested that the relation has importance in understanding the effect of taurine on membrane stabilisation and calcium fluxes in excitable tissues.²¹ Another study suggests that ethanolamine may be a product from degradation of membranes.²²

Amino acid concentrations in the cerebrospinal fluid have not previously been analysed in patients with chronic solvent intoxication syndrome. A few experimental studies of short term solvent exposed rats have been made regarding amino acid concentrations in the rat brain. In the brain of rats exposed to trichloroethylene and tetrachloroethylene no change in taurine concentrations was found.²³ That study, however, is not exactly comparable with our present study as our patients were exposed to various

solvents on a long term basis.

In conclusion, changes in the composition of cerebrospinal fluid may be associated with the diagnosis of chronic toxic encephalopathy. The clinical relevance of these findings are not clear but the amino acid changes may indicate membrane alterations that may be related to the exposure to organic solvents.

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