Quebec Cooperative Study of Friedreich's Ataxia

Taurine in Cerebrospinal Fluid in Friedreich's Ataxia

B. LEMIEUX, R. GIGUERE, A. BARBEAU, S. MELANCON AND D. SHAPCOTT

SUMMARY: *In a previous study we reported low values of taurine and aspartic acid in the CSF of patients with Friedreich's ataxia, when the results were compared to the literature. Further studies have revealed that unforetold difficulties with the advertised methodology of sequential multi-sample amino acid analysis were responsible for low values in the determination of these two amino acids in the small volumes necessary for CSF. A corrected method is presented. With the latter method the differences disappear for CSF taurine and aspartic acid, but they remain valid for the previously reported blood and urine values in Friedreich's ataxia. GABA levels are also normal in Friedreich's ataxia CSF.*

RÉSUMÉ: *Lors d'une étude antérieure nous avions rapporté des valeurs basses de taurine et d'acide aspartique dans le LCR de patients souffrant d'ataxie de Friedreich, lorsque nos résultats étaient comparés à ceux de la littérature. Des études subséquentes révèlent maintenant qu'il existait des difficultés imprévues dans l'application de la méthode recommandée, lorsque l'on emploi l'analyse séquentielle à plusieurs échantillons des acides aminés et que ces difficultés étaient responsables des valeurs basses obtenues pour ces deux acides aminés dans les volumes réduits disponibles pour l'analyse du LCR. Une nouvelle méthode corrigée est maintenant proposée. Avec cette méthode modifiée la différence entre les ataxiques et les contrôles dans le LCR disparaît pour la taurine et l'acide aspartique, mais il n'y a aucune modification des valeurs publiées dans les résultats concernant les acides aminés sanguins et urinaires chez les victimes d'ataxie de Friedreich. Les niveaux de GABA sont également normaux dans le LCR de patients ataxiques.*

From le Centre Hospitalier de l'Université de Sherbrooke; the Clinical Research Institute of Montreal and the Hôpital Sainte-Justine, Montreal.

Reprint requests for the entire supplement on Friedreich's ataxia (Phase Two, Part One) to: Dr. André Barbeau, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

In Phase One of the Quebec Cooperative Study of Friedreich's Ataxia we reported preliminary findings for the free amino acids in the cerebrospinal fluid (C.S.F.) of 15 patients with typical Friedreich's ataxia (group Ia) (Lemieux et al., 1976) and compared them to results from the literature for control C.S.F. (Scriver and Rosenberg, 1973; Plum, 1974). Our values for taurine were lower than the published control values. However, subsequent analysis of six samples of C.S.F. from normal subjects by our technique also showed low taurine levels. In view of the possible role of taurine in the pathophysiology of Friedreich's ataxia, we extended our work to determine the cause of the discrepancy between our control values and those reported in the literature. We report the results of amino acid analysis in 14 patients with typical Friedreich's ataxia, based on a modification of the method originally recommended for the determination of amino acids, using the Sequential Multi-Sample Amino Acid Analysis (operation manual for the technician TSM System. Technical publication No. TA-I-0233-10, 1973).

Except for the conclusions drawn from the previous results of taurine and aspartic acid levels in C.S.F., we will not discuss the other amino acids in C.S.F. or other physiological fluids, as these values were not changed by the technical modifications to be described

METHOD AND MATERIALS

(a) Patients:

This study was carried out on the CSF of patients previously studied in Phase One, except for two new patients. All 14 patients have typical Friedreich's ataxia (group Ia). However, on the 12 original patients, we obtained 5 new CSF samples and 7 were the original test samples previously analysed which had been stored at −20°C for less than one year.

(b) Controls:

First group (Original controls): CSF was obtained from six males (age 12 to 17) who had undergone lumbar puncture as part of a diagnostic study of their illness or as part of a study with pneumoencephalogram.

Second group (New controls): Using the same selection criteria, CSF from 3 males and 4 females of matched age were chosen for amino acid analysis. In all these control samples the biochemical parameters (protein, glucose, etc.) were within normal limits.

(c) Technique:

As the decrease in amino acid levels in CSF, in both controls and Friedreich's ataxia patients, was observed only for the first 2 or 3 amino acids eluted from the acidic neutral column, it was evident that we were dealing with a technical problem inherent in the type of apparatus used in the ion exchange chromatography of amino acids. This loss of amino acids could be due either to the deproteinisation technique, or more likely to the incomplete adsorption of sample to the cartridge. Two sets of experiments were designed to investigate this problem.

First experiment: (SSA (Sulfosalicylic Acid) deproteinisation of CSF). Different concentrations of SSA varying from 5 to 150 mg/ml CSF were used with and without previous lyophylisation of the sample and complete analysis of both standard solutions and CSF samples were carried out according to the previous method.

Second experiment: (Amino acid adsorption on the cartridge). A study of the volume of sample in relation to adsorption to the cartridge was made in order to determine the degree of retention of amino acids by the resin (Chromobead C-3). Solutions containing 10 nanomoles of a mixture of amino acids in a volume of 25, 50 or 100 μ l (with or without lyophylisation and reconstitution to the original volume) and also 200 μ l were added to the sample cartridges in the normal manner and analysed.

TABLE I

AMOUNTS OF SULFOSALICYLIC ACID (SSA) mg/ml OF CFS USED BY DIFFERENT AUTHORS AND THEIR VALUES FOR TAURINE

	AUTHORS	DEPROTEINISATION TECHNIQUE	SSA CONCENTRATION IN CSF mg/ml	TAURINE μM/l
1.	LAKKE and TEELKEN (1976)	10% SSA (7.1 v/v)	700 mg	7.5 ± 2.4
2.	MUTANI et al. (1974)	4 ml of 3.75% SSA per ml of CSF	150 mg	5.1 ± 0.5
3.	LEMIEUX et al. (1976)	9% SSA 1:1 v/v 0.5 ml of CSF	90 mg	8.9 ± 4.0
4.	LIAPPIS et al. (1977)	5% SSA 1:1 v/v	50 mg	7.7 ± 4.5
5.	DICKINSON and HAMILTON (1966)	150 μl of 15% SSA with 0.75 ml of CSF	30 mg	5.8 ± 1.8
6.	GJESSING et al. (1972)	10 mg SSA/ml	10 mg	6.8 ± 1.7
7.	SZILAGYL et al. (1974)	5 mg SSA/ml	5 mg	- -
8.	PERRY et al. (1975)	5 mg SSA/ml	5 mg	6.6 ± 1.7
9.	VAN SANDE et al. (1970)	5 mg SSA/ml	5 mg	5.3 ± 1.4

Current method for CSF analysis

In order to reduce to a minimum the volume of CSF for better adsorption on the cartridge, we have chosen the process of lyophylisation. CSF was collected by lumbar puncture (usually performed in the morning when the subjects were fasting) with a simultaneous plasma sample. 500 μ l of CSF was deproteinized with 500 μ l of 9% SSA, with 10 nanomoles of norleucine added as an internal standard. After centrifugation for 10 minutes at 6,000 r.p.m., the supernatant was lyophylized, and the residue was then dissolved in 200 μ l of sample buffer. The total volume (200 μ l) was added to the cartridge in the usual manner. The concentration of each of the ninhydrin positive substances is expressed in μ M/liter of CSF by comparison to a standard mixture of amino acids containing 10 nanomoles in a volume of 200 μ l.

RESULTS

1) SSA Deproteinisation:

Different concentrations of SSA did not alter the recovery from standard amino acid solutions added to cartridges, either for the first amino acids eluted, such as taurine, or for all other amino acids from the acidic or basic column. A review of the quantity of SSA used by different investigators for the deproteinisation of CSF is shown in Table I with the values for taurine using our new method.

2) Adsorption on the cartridge:

A fixed amount (10 nanomoles) of the first two amino acids eluted from the acidic/neutral column (cysteic acid and taurine) was dissolved in different volumes of sample buffer. The recorder peak areas obtained are shown on Table II. The loss of these amino acids is proportional to the volume of sample solution. There is no statistical difference in the recovery for solutions which had been lyophylised and reconstituted to the original volume.

New method of CSF amino acid determination

Table III shows the result, for control and Friedreich's ataxia patient groups of 23 amino acids analysed by the original and the new methods. There are no statistical differences with either methods for any amino acids. Taurine and aspartic acid in both our controls and Friedreich's ataxia patients were low with the original method, although not different from each other. The values are now higher, but are still not different from each other.

Table IV shows the individual values for taurine and aspartic acid in individual patients by the new method, including their plasma/CSF ratio. The mean and standard deviations are compared with current data from the literature. The apparent slight increase of taurine concentrations in CSF in Friedreich's ataxia could be due to different steps involved in the preparation of the CSF sample, or more likely to the Ion Exchange Chromatographic System which we employ. The aspartic acid

TABLE II

EFFECT OF SAMPLE VOLUME ON IDENTICAL AMOUNTS OF STANDARD AMINO ACID SOLUTIONS LYOPHYLISED AND REDISSOLVED IN VARYING VOLUMES OF SAMPLE BUFFER.

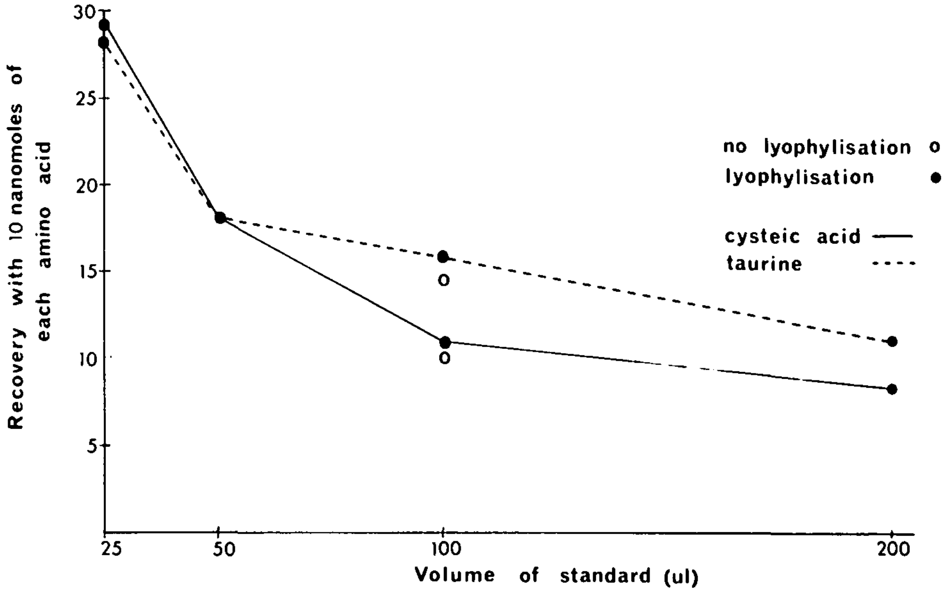

TABLE III

NEW AND ORIGINAL F.A. AND CONTROL VALUES (MEAN ± S.D. in µMol/l (CSF))

F.A. = Friedreich's Ataxia

AMINO ACIDS	ORIGINAL VALUES 15 F.A.	ORIGINAL CONTROL VALUES 6 SUBJECTS	NEW VALUES 14 F.A.	NEW CONTROL VALUES 7 SUBJECTS
Taurine	0.5 ± 0.7	0.2 ± 0.2	8.9 ± 4.2	8.9 ± 4.0
Aspartic Acid	0.7 ± 1.0	1.9 ± 0.8	3.4 ± 1.4	2.6 ± 2.4
Threonine	16.5 ± 5.8	16.1 ± 6.1	16.4 ± 5.0	14.0 ± 5.2
Serine	18.0 ± 4.8	20.3 ± 9.3	16.5 ± 8.4	21.0 ± 9.5
Asparagine	2.7 ± 1.4	3.6 ± 1.7	1.8 ± 1.3	2.1 ± 2.5
Glutamic Acid	18.3 ± 21.1	52.0 ± 37.5	69.1 ± 76.4	53.6 ± 46.2
Glutamine	290.4 ± 116.2	169.0 ± 90.8	156.6 ± 89.7	210.2 ± 215.1
Glycine	4.4 ± 1.7	5.9 ± 2.7	5.6 ± 1.6	6.1 ± 3.3
Alanine	23.9 ± 8.4	20.9 ± 6.9	22.8 ± 6.5	14.7 ± 9.7
Citrulline	0.4 ± 0.7	1.0 ± 1.0	0.5 ± 1.1	1.1 ± 2.8
α-Amino N-But. Ac.	2.2 ± 1.3	2.3 ± 1.3	2.6 ± 2.1	3.6 ± 3.4
Valine	14.7 ± 2.8	14.2 ± 4.2	17.5 ± 4.7	9.3 ± 5.5
Cystine	- -	- -	- -	- -
Methionine	2.1 ± 1.0	1.9 ± 0.8	1.4 ± 1.2	1.6 ± 0.8
Isoleucine	5.0 ± 1.6	6.5 ± 1.9	6.4 ± 1.7	4.3 ± 3.4
Leucine	17.9 ± 14.8	12.5 ± 3.7	14.5 ± 4.5	10.1 ± 7.0
Tyrosine	10.3 ± 5.4	10.4 ± 2.4	10.9 ± 4.4	6.8 ± 6.8
Phenylalanine	10.0 ± 4.6	9.5 ± 2.5	9.5 ± 4.7	7.2 ± 6.0
Tryptophane	- -	- -	- -	- -
Ethanolamine	10.4 ± 5.4	8.6 ± 4.5	8.3 ± 5.0	4.4 ± 5.6
Ornithine	3.8 ± 1.4	4.3 ± 3.1	7.5 ± 4.0	4.3 ± 3.4
Lysine	20.4 ± 6.6	13.4 ± 3.3	28.6 ± 8.3	17.4 ± 14.3
Histidine	10.8 ± 3.8	7.3 ± 4.2	14.1 ± 7.5	8.3 ± 6.6
Homocarnosine	2.4 ± 2.9	4.4 ± 1.8	3.9 ± 3.8	2.3 ± 2.3
Arginine	18.9 ± 5.8	16.6 ± 3.5	23.8 ± 9.5	16.5 ± 15.0

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TABLE IV

TAURINE AND ASPARTIC ACID IN 14 F.A. PATIENTS AND CONTROL in μmol/l
F.A. = Friedreich's Ataxia

	TAURINE			ASPARTIC ACID		
	PLASMA	CSF	PLASMA CSF RATIO	PLASMA	CSF	PLASMA CSF RATIO
SBL 2	149.4	4.8	31.1	43.9	3.5	12.5
SBL 3	36.5	6.4	5.7	10.9	4.8	2.3
SBL 4	42.6	10.3	4.1	17.8	-	-
SBL 5	44.1	7.2	6.1	9.2	3.9	2.4
SBL 6	30.6	14.3	2.1	10.1	3.9	2.6
SBL 10	59.7	17.0	3.5	8.3	3.1	2.7
SBL 11	50.8	8.1	6.3	10.6	2.4	4.4
SBL 14	158.6	8.9	17.8	62.3	3.0	20.8
SBL 15	125.3	17.6	7.1	62.4	5.3	11.8
SBL 16	6.7	6.5	1.0	23.7	2.3	10.3
SBL 17	8.3	4.7	1.8	10.0	2.6	3.8
SBL 18	37.0	9.8	3.8	7.6	5.8	1.3
SBL 22	196.3	5.4	36.4	44.3	3.6	12.3
SBL 23	70.3	4.2	16.7	15.2	3.1	4.9
MEAN ± S.D.						
14 F.A.	73 ± 60	8.9 ± 4.2	8.1	24.0 ± 20.3	3.3 ± 1.3	7.3
OUR CONTROLS	44 ± 29 (37 subjects)	8.9 ± 4.2 (7 subjects)	4.9	18.0 ± 7.7	2.6 ± 2.4	7.5
VAN SANDE (1970)		5.3 ± 1.4 (13 subjects)			2.9 ± 2.7	
GJESSING (1972)		6.8 ± 1.7 (19 subjects)			0.6 ± 0.3	
PERRY (1975)	56 ± 13 (77 subjects)	6.6 ± 1.7 (43 subjects)	8.5	2 ± 1	0.3 ± 0.3	6.7

value is comparable to that reported by van Sande (1970).

DISCUSSION

Deproteinisation with different concentrations of SSA with and without lyophylisation did not alter the quantitative analysis of amino acids on Ion Exchange Chromatography. We are not aware of any report on the effect of SSA concentration and lyophylisation on the CSF proteins. As the CSF protein content is about 200 times less than the plasma protein content, there is no need to utilize more than 10 μ-g/ml SSA. Moreover, the amount of SSA or the effect of lyophylisation should theoretically have interfered with all amino acids in the samples.

While carrying out the amino acid adsorption on the cartridge, Essner (1976) published his data on a fast automated chromatographic method for analysis of plasma amino acids. He showed that the cartridge resin did not quantitatively retain those amino acids first eluted from the column, including cysteic acid, homocysteic acid, taurine and phosphoethanolamine, if the volume of the sample exceeded 20 μ l. In reviewing the data of Perry and collaborators (1961, 1968, 1975), using a slow single column system, there are still some slight modifications between the values of amino acids reported and our own with the new method.

Using a fast sequential automated system (TSM), we had followed the instruction manual where it was explicitely mentioned: "upon aspiration, the sample amino acids are adsorbed on to the cartridge resin, excess fluid passing through. With the exception of cysteic acid, this adsorption is quantitative, provided that the total volume is under 0.5 ml". However, as we have shown here, the sample volume is critical for the efficient retention of cysteic acid and taurine, and it is evident that large volumes should not be used for CSF.

In 4 of the patients with typical Friedreich's ataxia, Dr. H. I, Yamamura of Tucson performed determinations of CSF GABA concentrations. No significant difference was found between the levels in these patients and those in several dozen controls (personal communication).

The results shown in Tables III and IV do not substantiate a decrease or an increase of taurine or aspartic acid in the CSF of Friedreich's ataxia patients, and our previous report could have been misleading due to unsuspected technical problems. However, this difficulty does not apply to the larger volumes studied in blood and urine, where our previous conclusions stand.

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