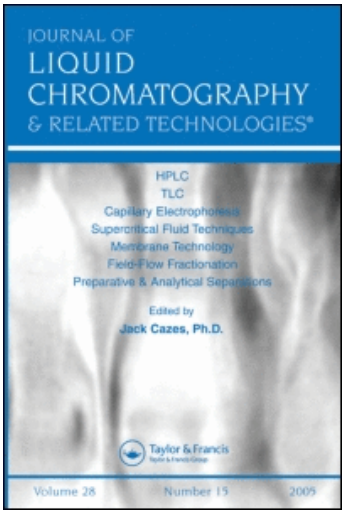

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Analysis of Taurine in Blood Plasma of Epileptic Patients Using an Improved Isocratic HPLC Method for Amino Acids

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ANALYSIS OF TAURINE IN BLOOD PLASMA OF EPILEPTIC PATIENTS USING AN IMPROVED ISOCRATIC HPLC METHOD FOR AMINO ACIDS

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ABSTRACT

An efficient, reversed-phase HPLC method is described in which 21 derivatized amino acids were isocratically separated in less than 90 minutes. An internal standard was used to improve precision and accuracy. The method, which separated taurine from α-, β-, and γ-aminobutyric acids, was used to quantify taurine levels in 83 blood plasma samples. A statistical comparison was made between taurine levels found in the plasma of epileptic and non-epileptic children.

INTRODUCTION

In the analysis of amino acids and aminosulfonic acids, the use of precolumn derivatization with o-phthalaldehyde (OPA, 1,2-benzenedicarboxaldehyde) in the presence of a thiol to form highly fluorescent substituted isoindoles has become fairly widespread (1-9). In 1982, Hill, et al. (4) characterized the

optimum experimental parameters for the precolumn derivatization reaction using OPA and ethanethiol (EtSH). In their report they noted that α-amino-n-butyric acid (α-ABA) and β-aminoisobutyric acid (β-ABA) co-eluted with taurine (2-aminoethanesulfonic acid). Other authors (7,8) also reported difficulties in quantifying taurine in biological fluids due to co-elution with either α-ABA or β-ABA. In a recent paper (10), we reported the use of a ternary solvent system (acetonitrile/tetrahydrofuran (THF)/aqueous phosphate buffer) and efficient 5- and 10-micron octadecyl-bonded column packing materials to separate α-ABA and β-ABA from taurine.

This report describes an improved version of the above procedure in which γ-amino-n-butyric acid (GABA) and hypotaurine derivatives have also been separated from taurine, α-ABA, and β-ABA. Hypotaurine has been suggested to be a precursor in the biosynthesis of taurine (11), and GABA is a known neurotransmitter (12). An internal standard, homotaurine, has been added to improve the precision and accuracy of the analysis of taurine by reducing dependence on injection methodology.

The chromatographic system was able to separate 21 amino acids, of which 16 were quantifiable, in less than 90 minutes. The analysis for taurine, α-ABA, β-ABA, GABA, and hypotaurine could be run in 60 minutes. An additional advantage of this method is the use of isocratic conditions, allowing its use in laboratories not equipped for gradient elution.

The method was used to quantify taurine levels in 83 blood plasma samples from epileptic and non-epileptic patients, and the

differences in levels found between groups was statistically analyzed.

MATERIALS

A Waters Associates Model 201 Liquid Chromatographic System equipped with a Model 6000A pump was used. The sample injector was a Rheodyne Model 7125 with a nominal 20 µl loop. The fluorescence detector was a Schoeffel/Kratos FS-970 Spectrofluoro Monitor with a standard 5 µl cell. The detector parameters used were: excitation wavelength 229 nm, emission cutoff filter 480 nm, time constant 0.5 sec, fine sensitivity setting 5.00 on the 1.0 µA full-scale range. Chromatograms were recorded using a two-pen, 10 mV electronic integrating recorder (Houston Instruments Omniscribe Model 5213-15) connected in parallel with a Hewlett-Packard Reporting Integrator, Model 3390A, on which the peak retention times and peak heights were reported. Quantitative measurements were based on the peak heights reported by the integrator.

The analytical column (IBM Instruments Part No. 8635308) was packed with 5-micron porous-spherical, octadecyl-bonded silica. Column dimensions were 250mm x 4.5mm i.d. The guard column (IBM Instruments Part No. 8634990) contained the same 5-micron packing, and had dimensions of 50mm x 4.5mm i.d. The column system generated approximately 22,000 theoretical plates for the total 300 mm of column length.

The column compartment was thermostatted at 27.5 $\pm$ 0.5 °C with a circulating water bath (Model T3, P.M. Tamson, Holland). Temperature was monitored by a Model 49TA digital thermometer (YSI

Scientific). The column jacket was a precisely grooved aluminum cylinder fabricated by the University of Connecticut Chemistry Department's machine shop. The column conditioning, described by Hill et al. (4), was carefully followed.

REAGENTS

Taurine, ethanethiol, and individual amino acids (reagent grade) were obtained either from Aldrich Chemical Co. or Sigma Chemical Co. Fluoropa brand o-phthalaldehyde was obtained from Pierce Chemical Co. Acetonitrile (UV), tetrahydrofuran (UV), and methanol (all of HPLC quality) were obtained from either Burdick & Jackson Laboratories or MCB Manufacturing Chemists, Inc. Water used to prepare the chromatographic mobile phase was first condensed from steam, then passed thru an organic removal cartridge, two mixed-ion exchange resins, followed by a 0.20 μm submicron filter (Nanopure 4-module system with pump, Sybron/Barnstead). The resulting water would qualify as reagent grade, Type I-ACS standard water having a specific conductivity greater than 10 megohms/cm. All solutions and samples involved in the liquid chromatographic separation were filtered thru 0.22 μm filters (Millipore Corp.) prior to use.

METHODS

Preparation of Solutions

Individual stock solutions (1.00×10^{-2} M) of each amino acid were prepared in 0.1F HCl. Appropriate volumes of each stock solution were accurately transferred to a 100 mL volumetric flask

and diluted to volume with 0.1F HCl. The resulting working standard contained the following amino acids, with concentrations (nanomoles/ml) indicated in parentheses: asparagine (100), serine (150), glutamine (200), histidine (150), 1-methylhistidine (20), citrulline (50), glycine (200), threonine (200), arginine (150), α-alanine (200), tyrosine (100), β-alanine (20), α-amino-n-butyric acid (50), taurine (100), β-aminoisobutyric acid (20), and γ-amino-n-butyric acid (10). The concentrations of amino acids in the working standard solution were similar to those values reported in human plasma (13). A 1.00×10^{-4} M solution of homotaurine served as the internal standard.

The derivatizing reagent was prepared just prior to use by adding 100 μL (83.1mg) of ethanethiol to 5.0 mL of OPA stock solution. This OPA stock solution was prepared by dissolving 1.00g of o-phthalaldehyde in 50 mL of methanol, and was protected from light and stored at 1°C when not in use. Because of the odor and volatility of ethanethiol (b.p. 35°C), all handling of these solutions was done in an efficient hood. The borate buffer was prepared by adding an excess of boric acid to 1.0 L of distilled water and heating to form a saturated solution. After cooling, the excess boric acid was filtered out, and the pH of the aqueous phase was adjusted to 9.5 with sodium hydroxide solution.

The tetrahydrofuran/acetonitrile/aqueous phosphate (8/12/80 % by volume) mobile phase was prepared as follows: the stock aqueous phosphate buffer was prepared by dissolving 14.10 grams of $NaH_2PO_4 \cdot H_2O$ and 36.76 grams of Na_2HPO_4 in reagent grade water and

diluting to one liter. The resulting aqueous buffer solution had a pH of 7.0. To prevent the precipitation of the phosphate salt in the tetrahydrofuran, it was found necessary to first mix 80 mL of tetrahydrofuran with 120 mL of acetonitrile and about 200 mL water. To this mixture was added 40 mL of the stock aqueous phosphate buffer. The resulting solution was then diluted to one liter with water. Upon dilution, the solvent system consisted of 8% tetrahydrofuran, 12% acetonitrile and 80% aqueous phosphate buffer. This diluted solvent system was calculated to be 24.8 mM in sodium ion and 14.5 mM in phosphate. It has been shown that control of phosphate concentration in the mobile phase is important in optimizing the separation of closely-eluting peaks (4).

Derivatization

Amino acid standards and samples were derivatized as follows: 1-20 nanomoles of each individual amino acid in 50-500 µL of solution were transferred to 2.0 mL volumetric tubes with an adjustable Eppendorf pipet equipped with sterile tips. Then 200 µL of homotaurine internal standard (1×10^{-4} M) was added, followed by 500 µL of borate buffer and 500 µL of OPA/EtSH derivatizing reagent, respectively, and the resulting solution was diluted to the 2.0 mL mark with reagent grade methanol and efficiently mixed. The solution was allowed to remain in the hood for 2 minutes, and then a 100 µL aliquot was injected into the Rheodyne injector, thus overloading the 20 µL loop.

Blood Plasma Sample Preparation

To accurately measure taurine and other closely-eluting amino acids, it is necessary to pay extra attention to the details of specimen collection, deproteinization, and the storage of biological samples. Special precautions were taken to avoid contamination of the physiological fluids with blood platelets, which contain many amino acids, including taurine, in high intracellular concentration. The blood samples for this study were prepared as follows: Sterilized polypropylene tubes (Falcon Plastics Part No. 2063) and sterilized Eppendorf pipet tips (Part Nos. 2234160-0 and 2234170-0) were used for transfer and collection of the physiological samples. All glassware was first washed with anti-bacterial detergent (Linbro 7X cleaning solution, Flow Laboratories, Inc., McLean, VA), then rinsed with a solution, 10% by volume, of nitric acid in deionized water, and finally rinsed thoroughly with micropore-filtered, deionized water.

The blood samples were collected in purple-top (EDTA-washed), sterile, vacuum-sealed tubes (Becton-Dickinson) obtained from the blood-drawing center at the University of Connecticut Health Center. Within 20 to 60 minutes of the time of the blood drawing, the blood sample was centrifuged for 5 minutes at 1800 rpm (Model TJ-6, Clinical Centrifuge, Beckman Instruments Inc.). Then, 500 µL of the clear upper portion (plasma) was transferred to a sterile polypropylene tube, being careful to keep the pipet tip a distance away from the red blood cells. Exactly 5.00 ml of reagent grade methanol was added to the plasma, and the resulting

solution was vortex-mixed for 15 seconds at a 7.0 setting on a vortex mixer (Model K-550-6, VWR-Vanlab). The solution was centrifuged again at 1800 rpm for 5 minutes. The methanol extract was then transferred to a new sterile polypropylene tube, taking care to exclude any solids, and stored at -18°C. At the time of analysis, the sample was allowed to come to room temperature, then a 500 µL aliquot of the extract was transferred to a 2 mL volumetric tube along with 200 µL of the internal standard. The derivatization procedure for the plasma sample was identical to that described for the amino acid standards.

RESULTS AND DISCUSSION

Development of Isocratic HPLC Method

Using the previously-reported (10) ternary solvent system, (tetrahydrofuran/acetonitrile/aqueous phosphate, 10/10/80 % by volume), the OPA/ethanethiol derivatives of γ-amino-n-butyric acid (GABA) and β-aminoisobutyric acid (β-ABA) were found to co-elute on the 5-micron column described. It was found that by adjusting both the THF/acetonitrile ratio and the aqueous phosphate concentration that β-ABA and GABA could be separated, while maintaining the separation of hypotaurine, taurine, and α-amino-n-butyric acid (α-ABA).

The separation of these five amino acid derivatives can be seen in Figure 1, in which the calculated capacity factors (k') for the peaks shown are: 5.2 (arginine), 10.4 (tyrosine), 11.5 (hypotaurine), 12.6 (α-ABA), 13.6 (taurine). 14.4

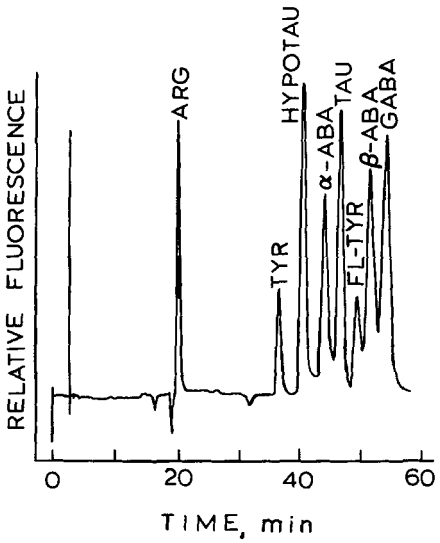

FIGURE 1. Isocratic Elution Profile of Certain of the OPA/Ethanethiol Derivatized Amino Acids. Conditions: 250 mm, 5-micron, octadecyl column, Solvent 12 % acetonitrile/8 % tetrahydrofuran/80 % aqueous phosphate buffer, Flow rate 1.0 mL/min, Column temperature 27.5°C.

(fluorotyrosine), 15.1 (β-ABA), and 15.9 (GABA). Attempts to decrease the k' values into the optimum k' range of 2 to 10 caused poorer resolution or co-elution of two or more components.

To improve the precision and accuracy by reducing dependence on the injection technique, it was desirable to identify an internal standard that eluted within a reasonable time period and was well separated from the other compounds. Several possibilities were investigated and homotaurine was chosen. The peak for the homotaurine derivative (k'=16.8) was found to elute directly after the peak due to GABA, and it was well separated

from other closely-eluting compounds. Homotaurine was found not to be present in the plasma of humans. Other compounds investigated as internal standard included: fluorophenylglycine, which had a retention time of about 120 minutes (k'=34), and homoserine, which eluted early in the chromatogram, and thus could not be used as an internal standard for taurine. The derivatized fluorotyrosine peak partially overlapped the peak for the β-ABA derivative (Figure 1). The fluorotyrosine derivative was also observed to be fairly unstable in the solvent system used.

Figure 2 shows the isocratic separation of 21 derivatized amino acids using this modified method. The separation took 90 minutes, and it was possible to quantify all the derivatized amino acids shown except for aspartic acid, glutamic acid, histidine and glutamine. As can be seen in the middle of the chromatogram, hypotaurine, α-ABA, taurine, β-ABA, GABA, and homotaurine are well separated (peaks 14-19 of Figure 2).

Figure 3 shows the chromatogram of the amino acid working standard described in the Methods section, with homotaurine as the internal standard. Calibration curves were established by taking 50-, 100-, 150-, and 200-μL aliquots of this working standard, adding 200 μL of the internal standard, derivatizing, and injecting as described above. Peak heights were measured by the reporting integrator, and a peak height ratio (amino acid peak height over internal standard peak height) was calculated for each amino acid peak and used to quantify the detector response. Table 1 shows the slopes, y-intercepts, and correlation coefficients, as

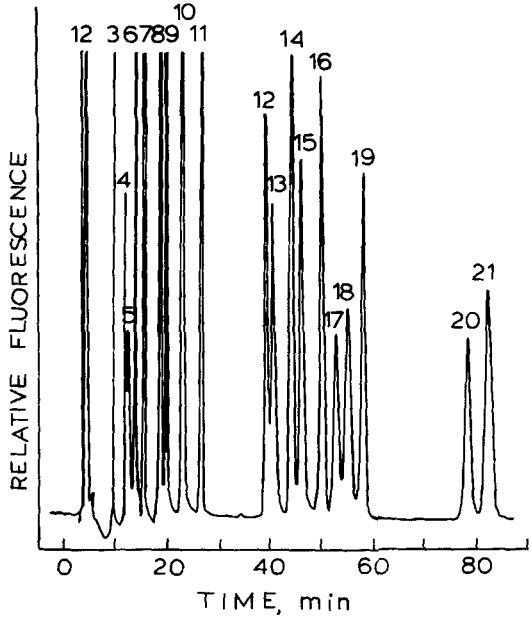

FIGURE 2. Isocratic Elution Profile of Twenty-One OPA/Ethanethiol Derivatized Amino Acids with Homotaurine Added as the Internal Standard. Conditions: 250 mm, 5-micron octadecyl analytical column and 50 mm, 5-micron, octadecyl guard column, Solvent 12 % acetonitrile/8 % tetrahydrofuran/80 % aqueous phosphate buffer. Flow rate 1.0 mL/min., Column temperature 27.5°C. Elution order of the OPA/Ethanethiol derivatized compounds corresponding to peak number is: (1) Aspartic Acid, (2) Glutamic Acid, (3) Asparagine, (4) Histidine, (5) Glutamine, (6) Serine, (7) Homoserine, (8) Glycine, (9) Threonine, (10) Arginine, (11) α-alanine, (12) Tyrosine, (13) β-alanine, (14) Hypotaurine, (15) α-amino-n-butyric Acid, (16) Taurine, (17) β-aminoisobutyric Acid, (18) γ-amino-n-butyric Acid, (19) Homotaurine, (20) Valine, and (21) Methionine.

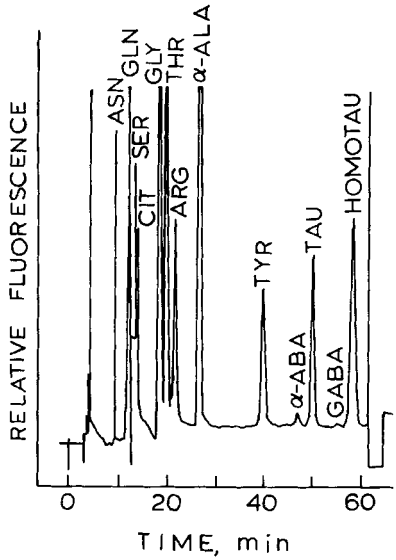

FIGURE 3. Isocratic Elution Profile of the OPA/Ethanethiol Derivatized Amino Acids Present in the Working Standard with Homotaurine Added as the Internal Standard. Experimental conditions same as Figure 2.

Table 1

Summary of the Calibration Data for Sixteen Amino Acids

Amino Acid	Range of Picomoles Injected	Linear Correlation Coefficient	Slope	y-Intercept
1. Asparagine	50-200	0.994	0.436	+0.044
2. Histidine	75-300	0.999	0.163	-0.303
3. Glutamine	100-400	0.999	0.064	-0.114
4. 1-Methylhistidine	10-40	0.998	0.595	-0.124
5. Serine	75-300	0.994	0.280	-0.039
6. Citrulline	25-100	0.999	0.608	-0.195
7. Glycine	100-400	0.995	0.211	-0.013
8. Threonine	100-400	0.995	0.208	+0.021
9. Arginine	75-300	0.999	0.245	+0.213
10. α-Alanine	100-400	0.996	0.208	-0.096
11. Tyrosine	50-200	0.999	0.202	-0.079
12. β-Alanine	10-40	0.999	0.099	-0.017
13. α-ABA	25-100	0.999	0.153	-0.029
14. Taurine	50-200	0.999	0.131	-0.087
15. β-ABA	10-40	0.999	0.077	-0.008
16. GABA	5-20	0.999	0.060	-0.007

determined by linear least squares, for the individual amino acid calibration curves (4 levels), as well as the overall range of sample sizes injected. Correlation coefficients of at least 0.999 were obtained for taurine, α-ABA, β-ABA, and GABA, indicating a linear response over the concentration ranges shown for these amino acids.

If fresh mobile phase was prepared daily, retention times of all of the derivatived amino acids were reproducible to within 3% in the worst case. In general, concentration measurements were found to be reproducible within 4% for within-the-day and 7% for between-the-day studies. In one case, methanol extracts of the same plasma sample were prepared at two different concentrations: one sample contained 0.50 mL of the plasma and the second 1.00 mL, both being diluted to 5.00 mL methanol. The taurine concentration was calculated to be 183 nmoles/mL in the first sample and 181 nmoles/mL in the second, a difference of about 1%.

Figure 4 shows a profile of the OPA/ethanethiol derivatives of amino acids extracted from the plasma of an epileptic patient. To detect components with low concentration, such as GABA, a larger volume (500 μL) of the methanol extract was derivatized. This generally gave an excessively high concentration of glutamine in the derivatized sample, which caused an overload in the fluorescence detector. Although partial separation of histidine, glutamine, and 1-methylhistidine derivatives has been achieved using this system, they could not be quantitatively determined in plasma samples because of the high concentration of glutamine relative to histidine and 1-methylhistidine.

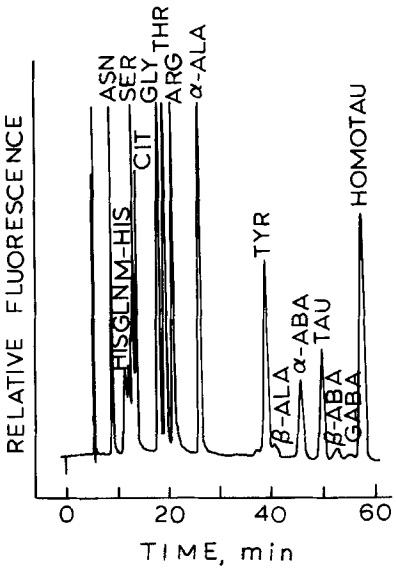

FIGURE 4. Isocratic Elution Profile of the OPA/Ethanethiol Derivatized Amino Acids Extracted from the Plasma of an Epileptic Patient with Homotaurine Added as the Internal Standard. Experimental conditions same as in Figures 2 and 3.

In the analysis of plasma samples, the mobile phase was changed in a step fashion to a mixture of acetonitrile/water (60/40% by volume) following the elution of the derivatized homotaurine peak in order to rapidly elute any of the more strongly-retained amino acid derivatives from the column.

Analysis of Taurine in Plasma

Once a nine-month period, 98 samples of blood plasma were analyzed. Fifteen of the samples were discarded, because they were either observed or believed to be contaminated by platelets of red blood cells. The remaining 83 samples were divided into

four groups as follows: Group 1 consisted of 26 plasma samples drawn from non-epileptic patients. Group 2 included 57 samples which were drawn from epileptic patients. Group 2 was sub-divided into Groups 3 and 4. Group 3 included 37 plasma samples drawn from epileptic patients whose seizures were judged to be under control by various drugs and the patients reported to be seizure-free for at least six months prior to the blood drawing. The remaining 20 samples, Group 4, were drawn from patients whose seizures were judged not to be under control at the time of blood drawing. Some Group 4 patients were reported as having from a few seizures per week up to 3 or 4 per day.

The mean taurine concentrations ($\pm$ standard deviations) found in plasma samples from the four different groups were as follows:

Group 1 (Non-Epileptic Patients)----- 112 $\pm$ 39 nmoles/mL

Group 2 (All Epileptic Patients)----- 142 $\pm$ 57 nmoles/mL

Group 3 (Controlled Seizures)-------- 137 $\pm$ 53 nmoles/mL

Group 4 (Uncontrolled Seizures)------ 158 $\pm$ 66 nmoles/mL

These results were statistically analyzed using the F-test and the t-test (14). Each individual group was then compared to Group 1. The results are summarized in Table 2.

When Group 2, all epileptic patients, was compared to Group 1, non-epileptic patients, the F-test showed no significant difference in the standard deviations at the 0.05 significance level (95 percent confidence). The t-test suggested a significant difference in the averages at the 0.05 and 0.02 significance level (95 and 98 percent confidence level). When Group 3, patients with controlled epilepsy, was compared to Group 1, the F-test showed no

Table 2

Summary of the Statistics Comparing the Different Groups of Patients.

Group#	$\bar{X}$	S.D.	n	F-test		t-test	
				F-calc.	F-table	t-calc.	t-table
one	112	39	26	2.14	2.46	2.43	2.01
two	142	57	57	no sig. diff. at 0.05 sig. level		a sig. diff. at 0.02 sig. level	
one	112	39	26	1.85	2.46	2.05	2.01
three	137	53	37	no sig. diff. at 0.05 sig. level		a sig. diff. at 0.05 sig. level	
one	112	39	26	2.86	2.46	2.77	*2.04
four	158	66	20	a sig. diff. at 0.05 sig. level		a sig. diff. at 0.01 sig. level	

$\bar{X}$ = mean value of the taurine concentration.
S.D. = standard deviation.
n = number of observations.
F-test compares the standard deviations for each group.
t-test compares the average concentrations of taurine for each group.
* requiring a special form of the t-test when the standard deviations are judged to be statistically different (14).

significant difference in the standard deviations at the 0.05 significance level. The averages of the taurine concentration (t-test) were found to be different at the 0.05 significance level. When Group 4, patients with uncontrolled seizures, was compared with Group 1, the F-test showed a significant difference between the standard deviations at the 0.05 significance level. A special form of the t-test required when the standard deviations

are judged to be significantly different (14) showed a significant difference between the mean value of the taurine concentration of the plasma of the two groups at the 0.01 significance level (99 percent confidence level). Thus, the plasma taurine concentrations for patients with uncontrolled seizures were found to be significantly higher than those for non-epileptic patients. A less significant difference was found for patients with controlled epilepsy.

On review of the above data, it was learned that there was no common dietary regimen imposed on the patients prior to the blood drawings. Since diet has been shown to affect taurine levels in biological fluids (15), variability of diet among patients may have had a substantial impact on the plasma taurine results reported above. Application of this improved HPLC method to additional blood samples drawn from patients on a common dietary regimen is recommended to ascertain whether there is any relationship between plasma taurine levels and occurence of epilepsy. The use of an autoinjector for the analysis is also recommended.

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REFERENCES

1. Hill, D.W., Walters, F. H., Wilson, T. D. and Stuart, J. D., "High Performance Liquid Chromatographic Determination of Amino Acids in the Picomole Range", Anal. Chem., 51, 1338 (1979).

2. Stuart, J. D., Wilson, T. D., Hill, D. W., Walters, F. H. and Feng, S. Y., "High Performance Liquid Chromatographic Separation and Fluorescent Measurement of Taurine", J. Liquid Chromatogr., 2, 809 (1979).

3. Lindroth, P. and Mopper, K., "High Performance Liquid Chromatographic Determination of Subpicomole Amounts of Amino Acids by Precolumn Fluorescence Derivatization with o-Phthaldialdehyde", Anal. Chem., 51, 1667 (1979).

4. Hill, D., Burnworth, L., Skea, W. and Pfeifer, R., "Quantitative HPLC Analysis of Plasma Amino Acids as Orthophthaldialdehyde/Ethanethiol Derivatives", J. Liquid Chromatogr., 5, 2369 (1982).

5. Jones, B. N., Pääbo, S. and Stein, S., "Amino Acid Analysis and Enzymatic Sequence Determination of Peptides by an Improved o-Phthaldialdehyde Precolumn Labeling Procedure", J. Liquid Chromatogr., 4, 565 (1981).

6. Godel, H., Graser, T., Foldi, P., Pfaender, P. and Furst, P., "Measurement of Free Amino Acids in Human Biological Fluids by High-Performance Liquid Chromatography", J. Chromatogr., 297, 49 (1984).

7. Thalmann, R., Comegys, T. H. and Thalmann, I., "Amino Acid Profiles in Inner Ear Fluids and Cerebrospinal Fluid", Laryngoscope, 92, 321 (1982).

8. Turnell, D. C. and Cooper, J. D. H., "Rapid Assay for Amino Acids in Serum or Urine by Pre-Column Derivatization and Reversed-Phase Liquid Chromatography", Clin. Chem., 28, 527 (1982).

9. Wheler, G. H. T. and Russell, J. T., "Separation and Quantitation of o-Phthalaldehyde Derivatives of Taurine and Related Compounds in a High Performance Liquid Chromatography (HPLC) System", J. Liquid Chromatogr., 4, 1281 (1981).

10. Eslami, M., Stuart, J. D. and Hill, D. W., "Separation of Orthophthalaldehyde/Ethanethiol Derivatives of Taurine and Closely Eluting Amino Acids by High Performance Liquid Chromatography", J. Liquid Chromatogr., 7, 1117 (1984).

11. Sturman, J. A. and Hayes, K. C., "The Biology of Taurine in Nutrition and Development", Chap. 9, in Advances in Nutritional Research, Vol. 3, Draper, H. H., ed., Plenum Publishing Co., New York, 1980, p. 233.

12. McLennan, H., "GABA Receptors", in The Role of Peptides and Amino Acids in Neurotransmitters, Lombardini, J. B. and Kenny, D. A., eds., Alan R. Liss, Inc., New York, 1981, Ch. 1.

13. Bremer, H. J., Duran, M., Kamerling, J. P., Przyrembel, H. and Wadman, S. K., Disturbances of Amino Acid Metabolism: Clinical Chemistry and Diagnosis, Urban & Schwarzenberg, Baltimore, 1981.

14. Miller, J. C. and Miller, J. N., Statistics for Analytical Chemistry, J. Wiley & Sons, New York, 1984, p. 52.

15. Huxtable, R. J. and Pasantes-Morales, H., eds., Taurine in Nutrition and Neurology, Vol. 139 of Advances in Experimental Medicine and Biology, Plenum Publishing Co., New York, 1982.