Journal of Chromatography, 164 (1979) 501—505
Biomedical Applications

CHROMBIO. 420

Note

Rapid liquid chromatographic determination of tryptophan, tyrosine, 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid

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(First received May 31st, 1979; revised manuscript received August 6th, 1979)

Tryptophan (TRP) and tyrosine (TYR) are precursors for the brain neurotransmitters serotonin (5-HT) and dopamine (DA), respectively. The major brain metabolite of 5-HT is 5-hydroxyindoleacetic acid (5-HIAA), while DA is predominantly metabolized to homovanillic acid (HVA). The measurement of these precursors and metabolites in cerebrospinal fluid (CSF) gives an indication of brain turnover of the neurotransmitters and is the most direct method available for assessing human brain neurochemistry.

TRP and TYR have been determined in CSF with fluorometric methods [1–4] and by using amino acid analyzers [5—7]. In general, the methods are time-consuming and require relatively large sample volumes (0.25—2.0 ml). Recently TRP has been quickly determined in 1—20 μl of CSF using a liquid chromatographic—fluorometric technique [8—10]. The acid metabolites, 5-HIAA and HVA, have been determined in CSF using fluorometry [11—19], gas chromatography—mass spectroscopy [20—23], and liquid chromatography coupled with either flow-through fluorometric [8—10, 24] or amperometric detectors [25].

We have developed a combined liquid chromatographic—fluorometric/amperometric method capable of determining all four of the compounds of interest within 9 min.

EXPERIMENTAL

Apparatus

Liquid chromatography was performed using an Altex 110 A pump (Altex Scientific, Berkeley, Calif., U.S.A.), a Waters Assoc. U6K injector and a μ-Bondapak C_{18} reversed-phase column (300 mm × 3.9 mm I.D., average particle size 10 μm) (Waters Assoc., Milford, Mass., U.S.A.). An Aminco Fluoromonitor (American Instrument, Silver Spring, Md., U.S.A.) was modified as previously described [8—10]. A further modification entailed cutting 2.0 × 20 mm slits

in the cylindrical flow-cell holder opposite the entrance and exit slits. In addition, the entire holder was dipped in 6 *M* HCl for several minutes, resulting in a dull grey finish. These changes reduced the background light level three to four fold. The amperometric detector consisted of a Bioanalytical Systems electrochemical controller (LC-2A), a carbon paste (silicon oil) working electrode, a Ag/AgCl reference electrode, and a Plexiglas thin-layer detector cell and reference electrode compartment (Bioanalytical Systems, West Lafayette, Ind., U.S.A.). A 51-μm spacer gasket was used, and the working electrode was set at + 0.8 V versus the reference electrode. The detector and reference cells were enclosed in a grounded faradaic cage. The fluorometric and amperometric detectors were connected in series, with the amperometric detector downstream.

Reagents

Standards were purchased from Sigma (St. Louis, Mo., U.S.A.). Stock solutions (10 mg/100 ml) were made up in distilled water with 0.1% ascorbate added. Diluted standards (0.1—5.0 ng/μl) were made up daily in 0.1 *M* HCl. The solvent system was prepared by mixing, for at least 1 h, 300 ml of glass-distilled methanol (Burdick and Jackson Labs., Muskegon, Mich., U.S.A.) with 1700 ml of pH 4.0, 0.01 *M* sodium acetate (adjusted with glacial acetic acid).

Methods

Usually 20 μl of unprocessed CSF, obtained by lumbar puncture, was directly injected into the system. Sample preparation consisted of centrifuging the CSF at 400 *g* for 10 min before injecting. The solvent system was delivered at a flow-rate of 1.5 ml/min. The compounds were quantitated by peak height measurements; single point standards were used as a linear response (peak height versus concentration) was observed over the working range.

RESULTS AND DISCUSSION

A chromatogram of TYR, TRP, 5-HIAA, and HVA standards is shown in Fig. 1. The retention times and absolute detection limits for the compounds are listed in Table I. Standards were determined at the 1—50 ng level with within-day coefficients of variation of from 2—11% (typically 5%). An unprocessed human lumbar CSF sample chromatogram is shown in Fig. 2. The column used

TABLE I

CHROMATOGRAPHIC AND DETECTABILITY DATA

Compound	Retention time (min)*	Detection limits (pg)**	
		Amperometric	Fluorometric
Tyrosine	2.4	60	350
Tryptophan	4.6	400	25
5-Hydroxyindole-acetic acid	6.2	10	45
Homovanillic acid	7.7	24	2000

*For chromatographic conditions see Experimental.

**Injected quantity giving a signal-to-noise ratio of 2.0.

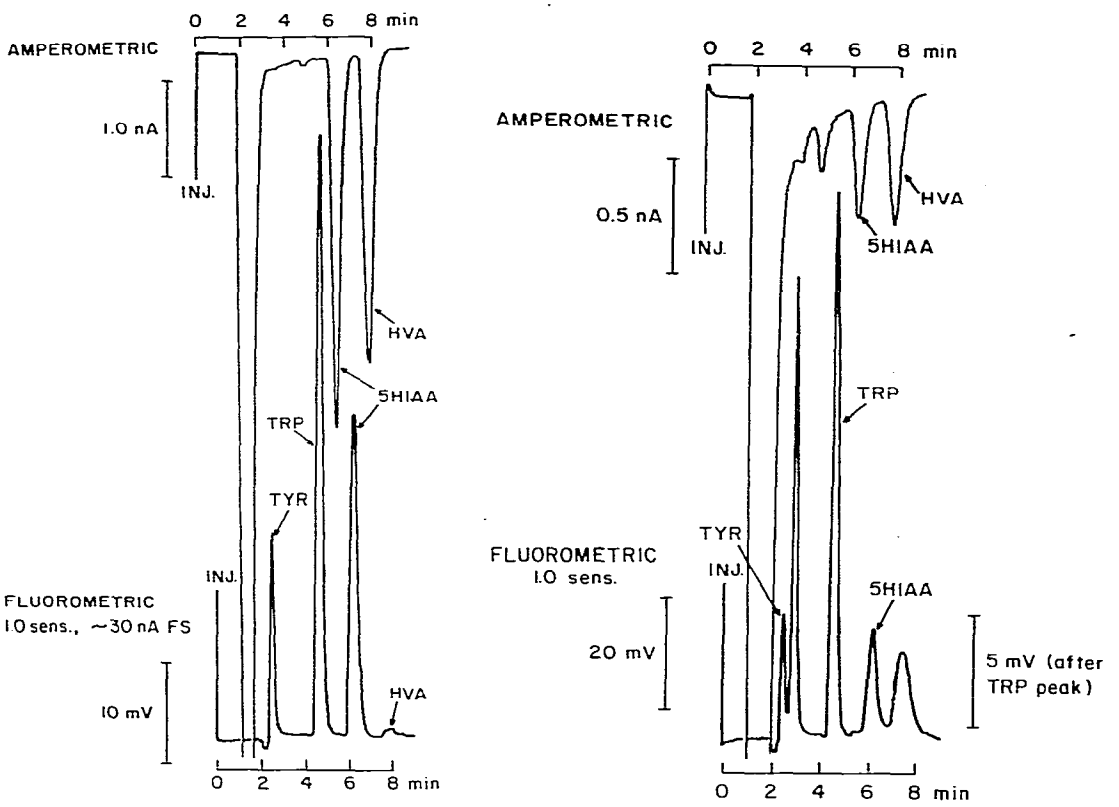

Fig. 1. Chromatogram of standards (25 ng TYR, 5 ng TRP, 5 ng 5-HIAA, 10 ng HVA) with amperometric and fluorometric detection. TYR was not usually separated from the amperometric solvent front. See Experimental for chromatographic conditions.

Fig. 2. Chromatogram of a 20-μl unprocessed lumbar CSF sample. Concentrations for the sample shown are: TYR, 1.04 μg/ml; TRP, 396 ng/ml; 5-HIAA, 32.6 ng/ml and HVA, 89.7 ng/ml. The fluorometric peaks immediately following the TYR and 5-HIAA peaks are unidentified. See Experimental for chromatographic conditions.

for the separations shown in Figs. 1 and 2 had been used to analyze more than 500 CSF and deproteinized plasma samples over a seven-month period. During that time the efficiency, or plate count (N), had decreased from ca. 2700 to ca. 1000. In a pooled human CSF sample the compounds were determined with the following coefficients of variation: TYR 6.7%; TRP 2.5%; 5-HIAA (fluorometric) 5.1%; 5-HIAA (amperometric) 2.9%, and HVA 10.7%. A standard addition study was performed by adding standards in amounts from 1—20 times the normal levels. The percent recoveries were: TYR 110 ± 5.9; TRP 93.7 ± 5.2; 5-HIAA 108 ± 6.1; and HVA 104 ± 4.0%.

The identities of the peaks observed in CSF were confirmed by chromatographing samples with solvent systems containing 10, 5 and 3% methanol or acetonitrile. The sample peaks always coeluted with the appropriate standard. The concentration ranges observed for the compounds have been similar to those reported in the literature.

A comparison between the fluorometric and amperometric determinations of 5-HIAA levels in 25 different human CSF samples is shown in Fig. 3. The methods were well correlated; usually 5-HIAA levels were taken to be the mean of the two methods.

A collaborative study is being undertaken comparing this method, a gas chromatographic—mass spectroscopic method, and a standard fluorometric procedure for determining 5-HIAA and HVA in CSF.

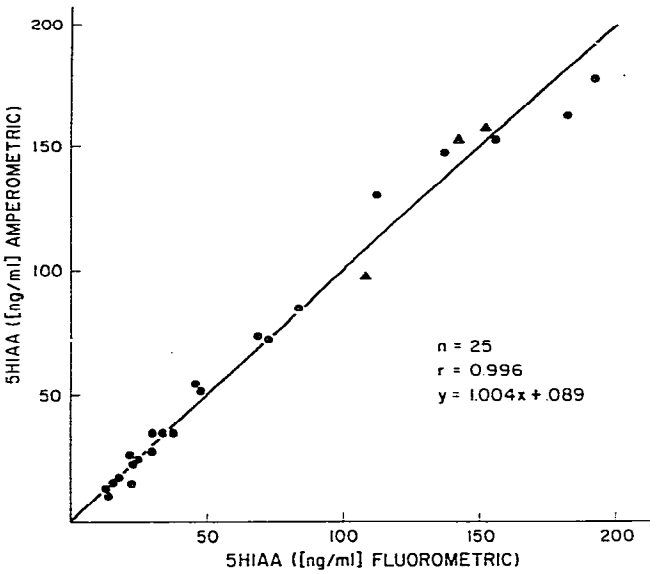

Fig. 3. The amperometric values for 5-HIAA plotted against the corresponding fluorometric values for 25 human lumbar CSF samples. Points plotted as ▲ are one-half the observed values.

ACKNOWLEDGEMENTS

We gratefully acknowledge the collaboration of B.A. Shaywitz and M.E. Kavanagh in these studies. We also thank Cs. Horváth for his encouragement. This research was supported by NIMH Clinical Research Center grant No. P50 MH 30929, NIH grant HD-03008, Children's Clinical Research Center grant RR0125, The William T. Grant Foundation, Mr. Leonard Berger, and the Solomon R. and Rebecca D. Baker Foundation.

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