A METHOD FOR THE DETERMINATION OF POLYAMINES IN CEREBROSPINAL FLUID

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1. Introduction

Data are rapidly accumulating which indicate that the determination of polyamine levels in physiological fluids may prove useful in diagnosing cancer and in evaluating the efficacy of various cancer treatment modes [1–3].

Methods are presently available for the determination of the polyamines putrescine, cadaverine, spermidine and spermine in urine [1–8], and some inroads have been made into their determination in serum [2]. However, though attempts have been made to analyze cerebrospinal fluid for polyamines [9], no definitive results have thus far been achieved due to the lack of an adequate method of analysis.

In the present study we have utilized a Durrum D-500 amino acid analyzer to quantitatively determine polyamine levels in cerebrospinal fluid. The method described allows for good sensitivity and reproducibility and is also applicable to urine and serum analyses.

2. Materials and methods

2.1. *Instrumentation*

A Durrum D-500 (Durrum Instrument Corp.) amino acid analyzer was used with the standard 5 mm path length flow cell.

2.2. *Resin*

Durrum DC-PA Cation exchange resin, a sulfonated polystyrene polymer with a bead diameter of 10±1 μm, was packed to a height of 8 cm in a stainless steel 1.75 mm internal diameter column.

2.3. *Buffers*

Buffer A: 0.067 M sodium citrate × $2H_2O$ (final Na^+ concentration = 0.20 M), pH 6.15±0.02. Buffer B: 0.30 M sodium citrate × $2H_2O$ and 1.50 M sodium chloride (final Na^+ concentration = 2.40 M), pH 4.68±0.02. Buffer C: 0.35 M sodium citrate × $2H_2O$ and 2.00 M sodium chloride (final Na^+ concentration = 3.05 M), pH 4.68±0.02. Five ml of thiodiglycol (Pierce Chem. Co.) and 1.0 ml of liquefied phenol (Matheson, Coleman and Bell) were added per liter of each buffer. Buffers were filtered through a Millipore filter (47 mm diameter, 0.45 μm pore size) before use.

2.4. *Chemicals*

Ninhydrin reagent solution [10] was purchased from Durrum Instrument Corp. The hydrochlorides of putrescine, cadaverine, spermidine and spermine were purchased from Calbiochem. ^{14}C-labeled polyamines were purchased from New England Nuclear.

2.5. *Cerebrospinal fluid (CSF)*

Samples of CSF from patients with central nervous

system tumors were obtained by lumbar puncture or from the ventricular system at operation. Control specimens of CSF from patients without neoplasm were collected during diagnostic lumbar puncture. Shortly after collection all samples were frozen at −20° C until analysis.

2.6. *Sample preparation*

Samples were prepared with hydrolysis as follows: 5 ml aliquots of CSF were acidified and lyophilized. The residue was reconstituted with 1 ml of 6 M HCl and hydrolyzed at 110° C for 14–16 hr. The hydrolyzed samples were lyophilized, reconstituted in 200 μl of distilled water and centrifuged at 8000 *g* for 10 min. 50 μl of the supernatant was utilized for the analysis. Samples were prepared without hydrolysis as follows: 5 ml aliquots of CSF were lyophilized, reconstituted in 200 μl of 4% 5-sulfosalicylic acid, kept at 0° C for 1 hr, and finally centrifuged at 8000 *g* for 10 min. 50 μl of the supernatant was utilized for the analysis.

2.7. *Amino acid analyzer parameters*

Elution and separation of the polyamines was accomplished in 70 min utilizing a 3-buffer system as follows: Buffer A was directed to the column for 27 min to elute the amino acids; Buffer B was then used for 23 min to elute putrescine, cadaverine, and spermidine; and finally Buffer C was used for 20 min to elute spermine. The buffer flow rate was 18.5 ml per hr and the ninhydrin flow rate was 9.5 ml per hr. Column temperature was held isothermally at 66° C and the column pressure generated during analysis was approximately 1600 pounds per inch^2 gauge. Full scale deflection on the recorder was set at 0.1 O.D. units at 590 nm. Following each analysis the column was regenerated with a solution of 0.2 M NaOH and 0.67 mM Na_2 EDTA and then equilibrated with Buffer A.

3. Results and discussion

We have previously described a method for the separation of polyamines in tissue extracts and urine [11]. This earlier method allows for the detection of as little as 25 pmoles of putrescine, 50 pmoles of spermidine and 100 pmoles of spermine with less than 10% coefficient of variation. Though the method is suitable for tissue extracts and urine, it is inadequate for serum and CSF where both a multi-buffer system (for the adequate separation of the polyamines from interfering substances) and high sensitivity (for the detection of the low levels of the polyamines present in these fluids) are required. A multi-buffer system could not be used with high sensitivity because of the marked column pressure variations that occured following buffer changes and which caused baseline shifts that interfered with the analysis.

The analysis of polyamines in CSF was facilitated by utilizing a resin which allows for buffer changes with almost no column pressure variation. Sensitivity, reproducibility, and linearity are the same as for the previously described method [11].

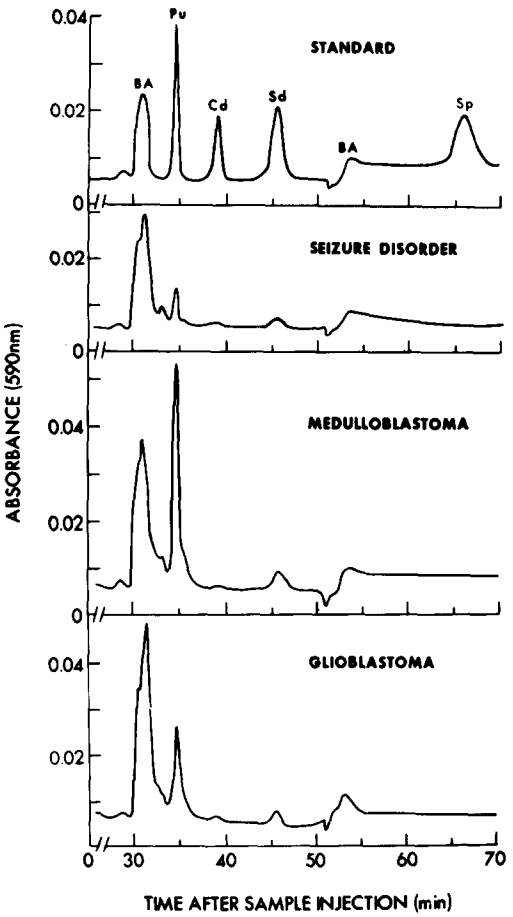

Fig. 1. Comparative amino acid analyzer chromatograms. BA = buffer artifact, Pu = putrescine, CD = cadaverine, Sd = spermidine, Sp = spermine.

Several CSF samples were divided into equal aliquots and analyzed following preparation with and without hydrolysis. The non-hydrolyzed aliquots showed significantly lower amounts of all of the polyamines, and all subsequent samples were hydrolyzed.

Recoveries of ^{14}C-labeled polyamines from the hydrolyzed preparation of the CSF samples were found to be 83% for putrescine, 67% for spermidine and 57% for spermine.

Fig. 1 shows a chromatogram of a standard mixture of 1 nmole of each of the polyamines, and chromatograms of 3 CSF samples. Putrescine and spermidine were found in all, and spermine in some, CSF samples analyzed; the identity of the peaks was corroborated by thin-layer chromatography of the dansyl derivatives. A peak eluting at the position of cadaverine was also noted in most samples.

From the 40 CSF samples so far analyzed it appears that patients with glioblastomas or medulloblastomas have increased putrescine and possibly spermidine concentrations in their CSF. These preliminary results indicate that polyamine determinations in CSF may be useful in the diagnosis and clinical evaluation of brain tumors.

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