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Letter to the Editor

Rapid high-performance liquid chromatographic determination of γ-aminobutyric acid and some other amino acids: application to rat brain

Sir,

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and may be associated with several neurological disorders [1,2]. Several high-performance liquid chromatographic (HPLC) methods for the determination of GABA in biological samples have been published; they often involve fluorimetric [3] or electrochemical detection [4], both using precolumn derivatization with *o*-phthalaldehyde (OPA). They are sensitive but generally not suitable for automation, and the OPA derivatives are less stable than the dansylated compounds.

This paper describes a method for amino acid determination using HPLC with fluorimetric detection after derivatization with dansyl chloride. The separation of GABA, alanine (Ala) and glutamine (Gln) is achieved in 15 min. Tissue preparation and dansylation are performed in less than 45 min. This rapid assay has been applied to rat brain areas.

EXPERIMENTAL

Reagents and chemicals

Stock standards solutions (1 mg/ml) of δ-aminovaleric acid (DAVA, internal standard), GABA, Gln and Ala (Sigma, St. Louis, MO, U.S.A.) were prepared in 0.1 *M* perchloric acid. Dansyl chloride (100 mg/ml) was dissolved in acetone and the solution was diluted to 1.25 mg/ml when required. All these reagents were stored at −20°C. $NaHCO_3$ (0.1 *M*), K_2CO_3 (0.1 *M*), acetonitrile, perchloric acid (0.1 *M*) and phosphoric acid (5 *M*) were of analytical reagent grade (Merck, Darmstadt, F.R.G.).

Apparatus

The HPLC system consisted of a Model 420 pump (Kontron) and a Rheodyne Model 7125 injection valve fitted with a 10-μl loop. The Nucleosil ODS (5-μm) column (150 mm × 4.6 mm I.D.) was protected by a Brownlee RP-18 precolumn

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(30 mm × 4.6 mm I.D.). The mobile phase was water–acetonitrile (72:28, v/v), degassed by sonication just before adjusting the pH to 3.0 with 5 *M* H_3PO_4. The flow-rate was 1.0 ml/min

The spectrofluorimeter (SFM25, Kontron) was used at wavelengths of 333 nm for excitation and 532 nm for emission.

Tissue preparation

Pieces of brain (10–15 mg) were cold disintegrated with ultrasound (sonicator) in 450 μl of 0.1 *M* perchloric acid to which 5 μg of DAVA (internal standard) were added.

After centrifugation (15 000 *g* at 4°C) for 15 min, the supernatant was filtered through a 0.45-μm Millex HV filter (Millipore, Bedford, MA, U.S.A.) and neutralized with 50 μl of 0.1 *M* K_2CO_3, then centrifugation for 2 min at 15 000 *g* was performed to eliminate the potassium perchlorate and 50 μl of supernatant were transferred into a glass tube for dansylation.

The standard mixtures were processed at the same time as brain samples: tissue was replaced with 50 μl of a mixture (Gln, Ala, GABA and DAVA each at 100 μg/ml) added to 450 μl of 0.1 *M* perchloric acid.

Dansylation

This procedure was performed essentially according to a recent method [5]. To the glass tube containing 50 μl of supernatant (see above), 50 μl of 0.1 *M* $NaHCO_3$ and 200 μl of dansyl chloride (1.25 mg/ml) were added. After vortex-mixing for several seconds, the tubes were placed in an oven at 90°C for 20 min, then the extract, diluted in the mobile phase, was injected into the HPLC system.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of a standard mixture (Gln, Ala, GABA and DAVA) injected directly after dansylation in the $NaHCO_3$ medium (Fig. 1a) or diluted in mobile phase (Fig. 1b), and a chromatogram of rat brain extract (Fig. 1c). Fig. 1 shows that the diluent ($NaHCO_3$ or mobile phase) is important for the profile and the retention times of the peaks. Therefore, all standard mixtures and extracts were diluted in the mobile phase and they remained stable for one day on ice in the dark.

The chromatogram of the standard mixture shows that GABA is well separated from alanine. Under our chromatographic conditions, only the pH modified the separation of GABA and alanine; at pH 3.0 the resolution was good whereas at pH 2.65 the two peaks coeluted. In this study, alanine determination was of no interest but it was important to isolate it from GABA. Ala was the only brain amino acid that disturbed the analysis.

GABA and glutamine determination was performed by the internal standard method. DAVA was chosen as the internal standard in brain extracts and gave

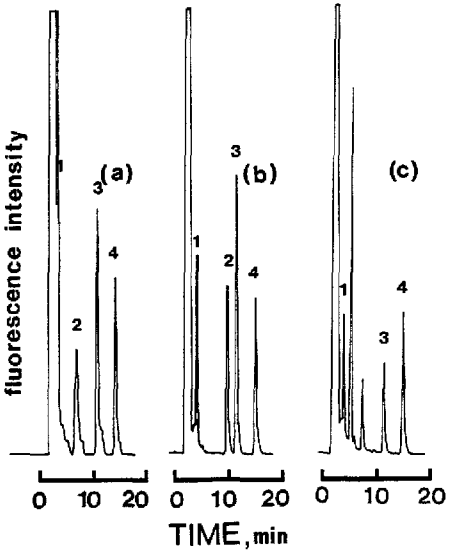

Fig. 1. Chromatograms of a standard mixture (25 ng injected), (a) diluted in $NaHCO_3$, (b) diluted in mobile phase and (c) extract from rat brain Chart speed, 0.2 cm/min Peaks 1 = Gln; 2 = Ala, 3 = GABA; 4 = DAVA.

good recoveries (85 ±19.7%; mean ± S.D., n = 20). The results are given in Table I and are comparable to those obtained by other workers [5,6].

The linearity of the dansylation reaction was tested for the four components (GABA, DAVA, Ala and Gln). The relationship between the amount dansylated (x) and peak height (y) was linear from 5 to 100 ng. The linear repression equations are the following: for GABA, $y = 61.33x - 3.773$ ($r = 0.999$); for DAVA, $y = 39.34x + 3.742$ ($r = 0.997$); for Ala $y = 48.04x - 3.847$ ($r = 0.999$); and for Gln, $y = 43.06x - 2.522$ ($r = 0.999$).

After successive dilutions of a dansylated standard mixture, the linearity of the fluorimetric response was verified in the range 500 pg–100 ng. In this study, the limit of detection was 25 pg injected with a signal-to-noise ratio of 2.

This method offers the advantages of speed and simplicity as far as the chro-

TABLE I

GABA AND Gln LEVELS

Results are means ± S.D. (n = 8), expressed in μmol/g wet tissue weight

Brain region	GABA (μmol/g)	GLN (μmol/g)
Cortex	2 0 ± 1.27	3.5 ± 0 79
Diencephalon	1.7 ± 0 54	3 2 ± 0.56

matographic analysis and sample preparation are concerned. Moreover, the good sensitivity allowed GABA to be determined in pieces of brain weighing less than 10 mg.

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