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Letter to the Editor

Ultra-rapid assay of brain γ-aminobutyric acid by liquid chromatography with electrochemical detection

Sir,

We have previously reported a rapid and simple method for the assay of γ-aminobutyric acid (GABA) in mouse brain, using liquid chromatography (LC) with electrochemical detection (ED) in combination with precolumn *o*-phthalaldehyde (OPA) derivatization [1]. Recently, a number of short reversed-phase columns packed with 3- or 5-μm particles have been developed for LC analysis of various biological materials. These short columns improve the resolution, sensitivity and analysis time. Thus, we have improved the analysis time and sensitivity of the previous method [1] for GABA assay by using a very short column (45 mm, 5 μm particle size). The method described here permits GABA assay within 1 min in one chromatographic run.

EXPERIMENTAL

Chromatography

The LC system consisted of a delivery pump (Model L-5000, Yanagimoto, Kyoto, Japan), a six-port injector (Model 7125, Rheodyne, Berkeley, CA, U.S.A.), and a C_{18}-IP Ultrasphere analytical column (45 mm × 4.6 mm I.D., 5 μm particle size, Beckman, San Ramon, CA, U.S.A.) protected by a guard column (Eicom prepak, 5 mm × 4.6 mm I.D., 7 μm particle size, Eicom, Kyoto, Japan). An electrochemical detector (Model VMD-501, Yanagimoto) with a graphite electrode (WE-3G, Eicom) was used at a voltage setting of +0.7 V versus an Ag/AgCl reference electrode. The temperature of the analytical and guard columns was controlled by a column jacket connected to a thermostatic water-bath (UC-65, Tokyo Rika Kikai, Tokyo, Japan) and maintained at 45°C.

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Measurement of GABA was based on the estimation of peak heights using an integrator (C-R6A, Shimadzu, Kyoto, Japan).

The mobile phase was a mixture of 0.05 *M* NaH_2PO_4, 0.1 m*M* Na_2EDTA, 46% (v/v) methanol and 4.6% (v/v) tetrahydrofuran. The solution was adjusted to pH 4.50–4.60 with phosphoric acid and filtered through a 0.45-μm membrane filter and degassed. The flow-rate was set to 3.6 ml/min, which yielded a pressure of 135 kg/cm^2.

Materials

Methanol and tetrahydrofuran (LC grade) and other chemicals were purchased from Wako (Tokyo, Japan). Water was double-distilled.

Amino acid standards

Stock standard solutions of amino acids (10 m*M*) were prepared with distilled water and stored at 4 °C for one month. Working standard solutions were from the stocks with a 0.05 *M* perchloric acid solution containing 0.1 m*M* Na_2EDTA every five days and stored at 4 °C.

Derivatizing reagents

The OPA–β-mercaptoethanol (BME) stock reagent was prepared essentially according to Allison et al. [2]. OPA (20 mg) was dissolved in 1.0 ml of ethanol, and 10 μl of BME and 9.0 ml of 0.1 *M* sodium tetraborate (pH 9.1) were added to the solution. This stock reagent was stored at 4 °C and could be kept for four days. The working OPA–BME solution was prepared by diluting 1 ml of the stock reagent with 9 ml of 0.1 *M* sodium tetraborate every 4 h and stored on ice during the experiment.

Derivatization

A total of 10 μl of a working standard solution or brain homogenate supernatant was thoroughly vortex-mixed with 40 μl of a working OPA–BME solution for ca. 2–3 s at ambient temperature in a 1.5-ml Eppendorf microtube. The derivatization time was 60 s, and 10 μl of the derivatizing mixture were injected onto the LC system.

Tissue preparation

Mice were killed by microwave irradiation (4 kW, 1.2 s, Model TMW-6402A, Toshiba, Tokyo, Japan). The brain samples were prepared according to a previous method [1].

Recovery, precision and detection limits

The recovery of GABA from tissue was measured in homogenates spiked with and without a known amount (10 μg) of GABA. Within-assay coefficients of variation (C.V.) were calculated from fifteen consecutive injections of the

same working standard solution. The detection limits of GABA, based on a signal-to-noise ratio of 3, were determined by injections of diluted working standard solutions and diluted tissue sample solutions.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of OPA–BME derivatives: (A) blank reagent; (B) a standard mixture of five amino acids; (C) striatum extract; (D) hypothalamus extract of mouse brain. Under the present instrumental and chromatographic conditions, the retention time of GABA was only 0.65 min. The GABA peak could be completely resolved from the solvent front and adjacent amino acids. No endogenous compounds interfering with the GABA peak were detected. Because no peaks were eluted after the GABA peak, the analysis was completed within 1 min.

The recovery of GABA was $101.5 \pm 1.7\%$ (mean $\pm$ S.D., $n = 10$). The within-assay C.V. was 1.9% for GABA. The linearity of the detector response was verified in the range from 30 pg to 100 ng of GABA. The detection limit for GABA in a diluted standard solution was 15 pg per injection (10 μl), but the detection limit for GABA in a diluted tissue sample solution was ca. 60 pg per injection (10 μl).

The retention time (min) of the main amino acids that may present in brain tissues were as follows: His, 0.193; Asn, 0.202; Cys, 0.203; Gln, 0.220; Arg, 0.222; Asp, 0.225; Ser, 0.235; Tau, 0.260; Orn, 0.263; Glu, 0.277; Gly, 0.295; Thr, 0.297; Lys, 0.310; Tyr, 0.433; Ala, 0.460; GABA, 0.647; Met, 0.842; Trp, 1.078; Phe, 1.215; Leu, 2.173.

In the present procedure, it is important to separate the GABA peak from

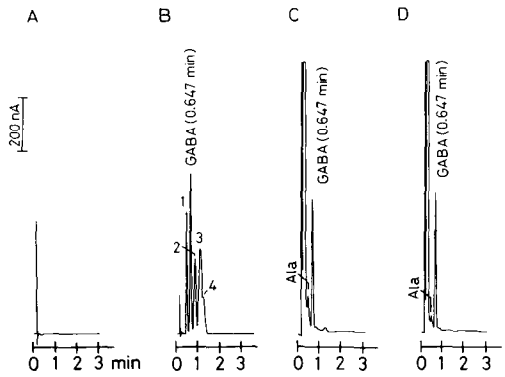

Fig. 1. Chromatograms of OPA–BME derivatives. (A) Blank reagent; (B) standard mixture of five amino acids (200 pmol each); (C) striatum extract (equivalent to 115 μg wet tissue); (D) hypothalamus extract (35 μg) of mouse brain. See Experimental for chromatographic conditions. Peaks: 1 = Ala; 2 = Met; 3 = Trp; 4 = Phe.

the Ala peak, because Ala is present in sufficient amounts in brain tissues to cause significant interference. For a clear separation of these two peaks, the pH of the mobile phase was the most effective of the four possible parameters (pH, column temperature, and methanol and tetrahydrofuran content) to adjust.

In conclusion, the present method, which is more rapid and more sensitive than our previous method [1] and those described by others [3–5], is suitable for the routine assay of brain GABA.

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