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Note

Determination of γ-aminobutyric acid in rat brain using an isotachophoretic analyzer

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γ-Aminobutyric acid (GABA) has been known to be present in considerable amounts in the brains of animals. It has been implicated as a major inhibitory neurotransmitter.

The determination of this compound has been achieved by using an amino acid analyzer, automated high-performance liquid chromatography [1], ion-exchange liquid chromatography after reaction with *o*-phthalaldehyde [2], enzymatic methods [3—5], and thin-layer electrophoresis [6]. GABA has been used as a terminating electrolyte [7] for the determination of other amino acids on an isotachophoretic analyzer, but isotachophoresis has never been used for the determination of GABA in biological samples because a good terminator for detecting GABA had not been found. We devised a new simple method for detecting GABA in rat brain by using an isotachophoretic analyzer [8—12].

EXPERIMENTAL

Male Wistar strain rats weighing 200 g were killed by decapitation, then the brain was removed, blotted, weighed and used immediately for the estimation of GABA. The brain was homogenized with 4 volumes of 1.25% sulfosalicylic

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acid and centrifuged at 1400 *g* for 10 min. The supernatant was applied to a column containing 10 ml of Diaion SK-1 (H^+-form of sulfonated cation exchanger, 100 mesh, Mitsubishi Kasei Co., Tokyo, Japan), washed with deionized water and eluted with 2 *N* ammonia. The eluate was dried under reduced pressure and aliquots of the residue were analyzed on an isotachophoretic and amino acid analyzer (Hitachi Model 835 liquid chromatograph).

Apparatus

The capillary apparatus was a Shimadzu IP-1B isotachophoretic analyzer (Shimadzu, Kyoto, Japan). The determination of GABA in rat brain was carried out in a capillary tube (20 cm × 0.5 mm I.D.) maintained at a constant temperature of 20°C. The detector cell had an I.D. of 0.5 mm and a length of 0.05 mm. The migration current was 100 μA. The leading electrolyte was 0.01 *M* potassium acetate in 0.02% polyvinyl alcohol, titrated with 17 *N* acetate to pH 4.5. The terminating electrolyte was 0.01 *M* carnitine chloride. The chemicals used were of analytical grade.

RESULTS AND DISCUSSION

The fraction collected containing GABA described under Experimental contained all amino acids. Therefore, the analytical conditions for detecting GABA without interference from other amino acids were studied. At first, the following analytical system was used for detecting GABA as an anion. The leading electrolyte was 0.01 *M* hydrochloric acid and β-alanine (pH 3.1). The terminating electrolyte was 0.01 *M* basic amino acids (lysine, arginine and histidine). However, GABA and the terminating electrolyte were detected in the same zone due to the fact that they have almost the same potential gradient. Accordingly, we could not detect GABA as an anion under these conditions. We then tried to detect GABA as a cation. The leading electrolyte was the same as that described under Experimental. Adenine was used as the terminating electrolyte. Although GABA could be detected as a cation under these conditions, we did not obtain good results because the potential gradient difference between adenine and GABA was very small. Therefore, another terminating electrolyte, carnitine, was used. In this analytical system, GABA, the terminating electrolyte and other basic amino acids were well separated as shown in Fig. 1. The acidic and neutral amino acids could not be detected.

Fig. 1B shows an isotachophoretic run of the brain sample, and Fig. 1C that of a mixture of the brain sample and authentic GABA. The zones of GABA in the brain sample and authentic sample as shown in Fig. 1C just overlapped each other and were elongated.

The standard curves drawn by plotting zone length against concentration of authentic GABA under the analytical conditions described above are shown in Fig. 2. The slope of the curve for authentic GABA was linear from 0 to 200 nmole. The recovery curve of GABA obtained after treatment with the column of Diaion SK-1 as shown in Fig. 2 (B) was slightly less than that in Fig. 2 (A), but the curve was also linear.

The recovery of GABA after treatment of Diaion SK-1 was about 85—95%. It was possible to detect 1 nmole of GABA in biological samples by using an

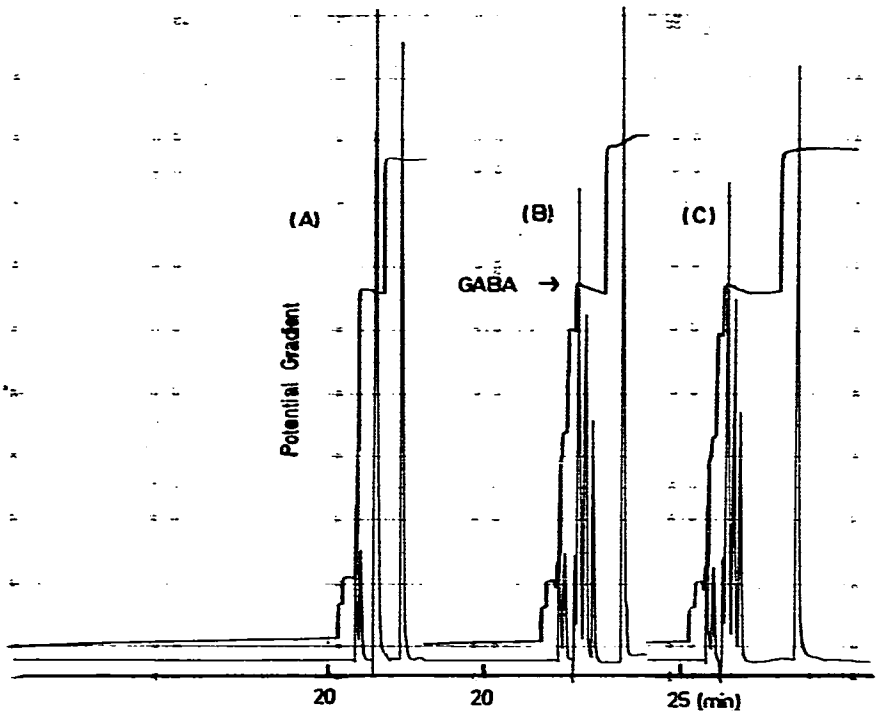

Fig. 1. Isotachophoretic runs of authentic GABA (A), brain sample (B) and a mixture of authentic GABA and brain sample (C). The leading electrolyte was 0.01 *M* potassium acetate and acetate, pH 4.5 (containing 0.02% polyvinyl alcohol). The terminating electrolyte was 0.01 *M* carnitine chloride. The migration current was 100 μA.

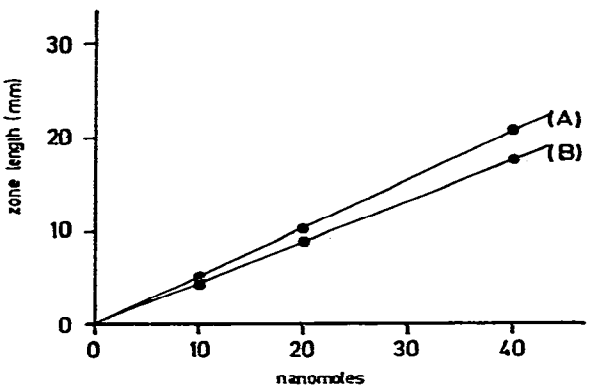

Fig. 2. Standard curves of authentic GABA (A) and GABA obtained after treatment of Diaion SK-1 (B). Analytical conditions as in Fig. 1.

isotachophoretic analyzer under the analytical conditions described in the Experimental section.

The comparison of the determination of GABA in rat brains using an isotachophoretic and an amino acid analyzer is shown in Table I. These two methods gave almost the same values. The results determined by using the isotacho-

TABLE I

COMPARISON OF GABA CONTENTS IN RAT BRAIN DETERMINED BY ISOTACHOPHORESIS AND AMINO ACID ANALYZER

Sample	GABA content (μmole/g wet weight)	
	Isotachophoretic analyzer	Amino acid analyzer
1	2.78	3.02
2	2.16	2.40
3	2.01	2.20
4	1.66	1.76
5	1.83	1.88

phoretic method described above for GABA levels in rat brain agreed well with earlier reports [1—5, 13]. This result shows that this method can be adequately utilized for the quantitative estimation of GABA in brain samples. The determination of GABA using the isotachophoretic analyzer presented here is simpler than with an amino acid analyzer, and should be very useful for determining GABA in rat brain.

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