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A rapid method for the quantitative analysis of γ-aminobutyric acid in hypothalamus homogenates by ligand-exchange chromatography

Transmission of neural impulses has been speculated to be regulated by γ-aminobutyric acid (GABA), which serves as an inhibitor of the process[1,2]. Therefore, the quantitative determination of this amino acid has become of importance for the elucidation of the role of GABA in the mechanism of neuronal transmission. Currently, there are three techniques that are widely used to measure quantitatively this constituent in tissue extracts or physiological fluids. These are paper chromatography coupled with fluorimetry[3], which is an extremely slow analytical procedure, ion-exchange chromatography[4,5], which provides for the elution of GABA from a typical amino acid analyzer, and a coupled enzyme assay in which γ-aminobutyric-glutamine transaminase and succinic semialdehyde dehydrogenase are used[6,7]. GABA elutes with the neutral amino acids by ion-exchange chromatography and with an elution time of 2.5–14 h[4,5], depending on the instrument used; hence this technique, although having the advantage of automation and ability to resolve quantitatively numerous other physiological components, has not been widely used to measure GABA in physiological fluids.

In this laboratory, we have used ligand-exchange chromatography[8] for the analysis of hypothalmus extract for GABA and have developed a method for the elution of GABA in 15 min from a cation-exchange resin equilibrated with zinc ions.

Experimental

Homogenates of hypothalmus tissue from albino rats (Sasco, Inc., Omaha, Nebr.) were prepared by the method of TALLAN[9], except that the final sample was dissolved in 55 m*M* acetate buffer of pH 3.1 just prior to analysis.

Amino acid analyses were performed in a Hitachi–Perkin-Elmer KLA-3B amino acid analyzer equipped for the analysis of protein hydrolyzates. Samples for the analysis of GABA were placed in a 0.9 × 18 cm column of resin (Hitachi–Perkin-Elmer 2612) equilibrated at 60° with buffer of pH 5.15 that contained, per liter, 47.0 g of anhydrous sodium acetate, 11.0 ml of glacial acetic acid, 0.22 g of anhydrous zinc acetate and 4 ml of 25% BRIJ[8]. The column was eluted with the same buffer at a rate of 90 ml/h and a fluid pressure of 5 kg/cm^2. The eluant stream was monitored for ninhydrin-positive components as described previously[8].

Results and discussion

The separation of GABA from other amino acids was accomplished by ligand-exchange chromatography. As shown in Fig. 1, this amino acid eluted from the basic column after the acidic and neutral amino acids and just prior to tyrosine. Under the conditions described, the elution time for GABA was 13 min. Although 60 min was required for the elution of arginine from the chromatographic column, the sequential operation of two identical columns reduced the sample analysis time to 30–35 min per sample, including column regeneration. Hence the sample analysis time by this technique was limited by the number of chromatographic columns that were used.

When known amounts of GABA were added to standard amino acid mixtures,

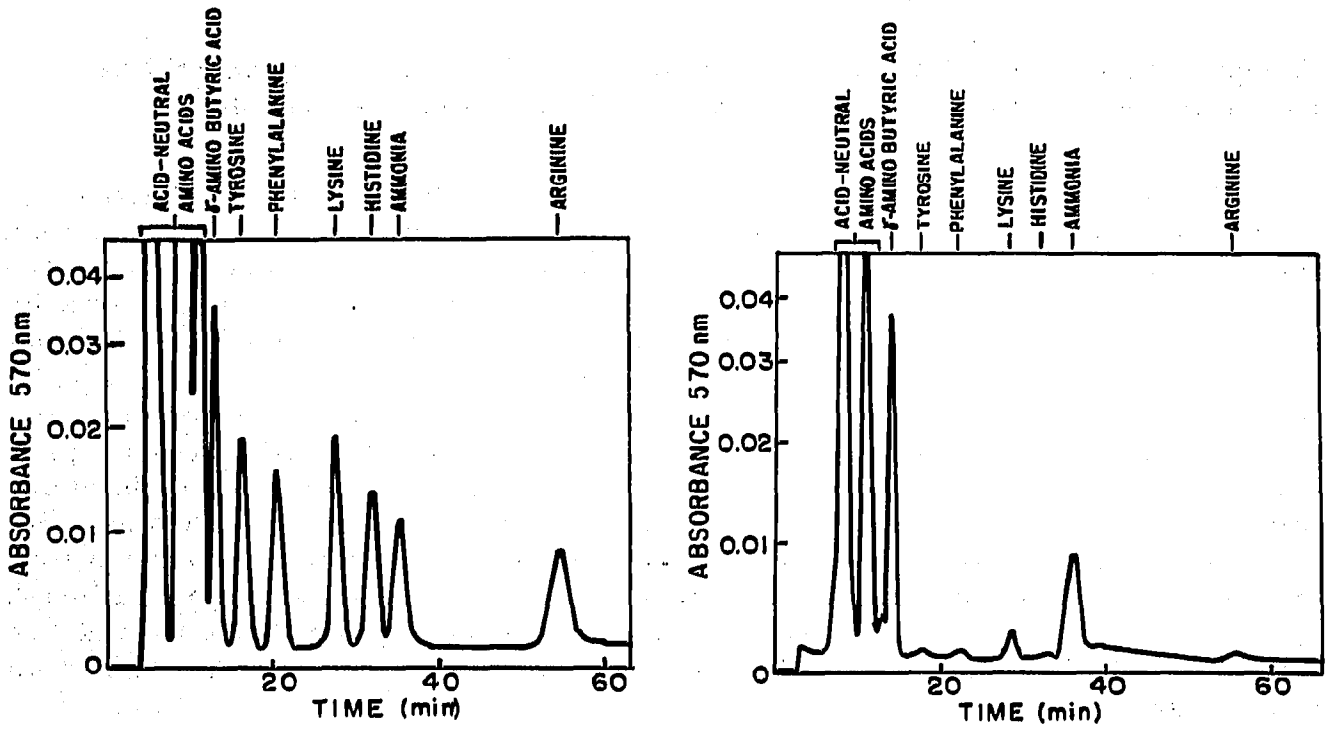

Fig. 1. Elution of amino acid standard mixture (50 μmoles of each residue) from a 0.9 × 18 cm column of cation-exchange resin equilibrated with 1 mM zinc ions at pH 5.15. The trace shows the absorbance at 570 nm. Full-scale expansion was 0–0.2 absorbance units.

Fig. 2. Elution of free amino acids from rat hypothalmus homogenate. Conditions were as in Fig. 1.

the peak area was found to be linear with GABA concentration and the reproducibility of any single determination using a standard solution of GABA was found to vary from 1 to 2%.

Fig. 2 shows a typical chromatogram of rat hypothalmus homogenate. Notable differences between this chromatogram and that shown in Fig. 1 are the relatively small amounts of basic amino acids and especially tyrosine. The marked lack of tyrosine only serves to improve the quantitation of GABA. Likewise, the lack of leucine and isoleucine in the samples decreased the ninhydrin-chromogen extinction in the trailing edge of the acidic–neutral band eluting from the basic column (Fig. 2), which also improved quantitation of GABA in brain homogenates.

Table I shows the GABA levels of six rats as determined by this method. It

TABLE I

γ-AMINOBUTYRIC ACID LEVELS IN RAT HYPOTHALMUS EXTRACTS

Technique	*μmoles GABA/g tissue*[a]	*Standard deviation*
Ligand exchange	3.17	0.34
Ion exchange[4]	3.06	0.55
Fluorometric[10]	3.23	0.20

[a] Wet weight.

can be seen that the precision of the technique was well within the normal deviation of GABA levels in rats. Also, the values obtained by this technique agree well with those obtained by other methods.

Finally, the technique provides a method for the detection of amounts of GABA as low as 5–10 nmoles when the photometer signal is set at its maximum sensitivity.

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