DETERMINATION OF GAMMA - AMINOBUTYRIC ACID BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

D. Qujeq

Department of Biochemistry and Biophysics, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

Abstract - Gamma-aminobutyric acid is the main inhibitory amino acid neurotransmitter in several areas of the brain. Measurement of gamma-aminobutyric acid in the brain will help elucidate its metabolic role and diagnostic value. The liquid chromatography (LC) system consisted of a delivery pump. Male rats (150-250g) were used in this study. The brain was rapidly removed from decapitated animals. The chromatograms showed satisfactory resolution and symmetrical peak shapes, both with the standard and tissue extracts. The retention time was 4.60 min. It was constant in repeated analyses and exactly the same for the standard and tissue samples.
Acta Medica Iranica 36 (2): 102 - 105; 1998

Key words: GABA, HPLC, neurotransmitter

INTRODUCTION

There is now good evidence to suggest that gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter substance in vertebrate as well as invertebrate CNS (1). GABA is synthesised in the CNS from L-glutamate by decarboxylation catalysed by glutamate decarboxylase. GABA is degraded by trans-amination catalysed by GABA transaminase (2). Levels in centeral nervous system may reflect altered GABA metabolism in various neurologic and psychiatric diseases. Measurements of GABA in tissues will help elucidate its metabolic role and diagnostic value (3). Numerous assays exist for the measurement of GABA concentrations in brain and other tissues: radioreceptor assay (4), the enzymic "GABA-ase" method (5), thin layer chromatography with fluorescence detection (6), and high performance liquid chromatography (HPLC), followed by electrochemical (7) or spectrofluorimetric detection (8). GABA is an important transmitter in central nervous system, since it is important to have a rapid and sensitive method for the determination of GABA in discrete brain regions.

MATERIALS AND METHODS

GABA, Methanol, Tetrahydrofuran, NaH_2PO_4 and Na_2EDTA were obtained from Sigma. Water was double-distilled. HPLC was performed on a delivery pump (Shimazu). Chromatograph composed of two series 10 pump, equipped with a loop injector valve with a 50 μ loop and a solvent programmer model series 10 LC controller. The analyses were performed on a C18-Ip ultrasphere analytical column (45 mm $\times$ 46 mm ID, 5UM particle size, Beckman). An electrochemical detector (Model VMD-501, Yanagimotto) with a graphite electrode (WE-3G, Eicom) was used at a voltage of +0.7V versus on Ag/AgCl reference electrode.

Male rats weighing 150-250 g were used. The animals were kept in Makrolon cages at an ambient temperature of 24-26°c and fed on altromin standard food. Rats were killed by microwave radiation. The brains were rapidly removed from decapitated animals, then they were rapidly dissected on ice-cooled glass plates, and homogenized immediately in 2 ml 80% ethanol. The o-phthalaldehyde - B - mercapto- ethanol (OPA-BME) stock reagent was prepared essentially according to the method of Allison (9). OPA (30 mg) was dissolved in 2.0 ml of ethanol, and 20μl of BME and 10.0 ml of 0.1 M sodium tetraborate were added to the solution. The working OPA-BME solution was prepared by diluting 2ml of the stock reagent with 10 ml of 0.1M sodium tetraborate every 5h and stored on ice during the experiment. A total of 20μl of a working

standard solution or brain homogenate supernatant was thoroughly vortex-mixed with 50μl of a working OPA-BME solution for 3-5 sec at ambient temperature in a 1.5ml eppendorf microtube. The derivatization time was 80s , and 20μl of the derivatizing mixture were injected into the LC system. Overall recovery was gauged by comparison with data obtained by HPLC analysis (assuming 100% recovery of derivatives) of the residue obtained when standard solution of authectic compounds equal in amount to those added to striatal homogenate aliquots were taken to dryness directly. Within-assay coefficients of variation (C V) were calculated from twenty consecutive injections of the same working standard solution. The detection limits of GABA based on a signal- to - noise ratio of 4, were determined by injection of diluted working standard solutions and diluted tissue sample solutions.

RESULTS

Figure 1 shows typical chromatograms of OPA-BME derivative: (A) blank reagent; (B) standard of GABA; (C) rat brain tissue samples. The chromatograms showed satisfactory resolution and symmetrical peak shapes, both with standard and tissue extracts. Under the present instrumental and chromatographic condition, the retention time of GABA was 4.60 min.

The recovery of GABA was 105.6 + 1.3% (mean + S.D, n= 12). The within assay C V was 2.1% for GABA. The linearity of the detector response was verified in the range from 20pg to 120 ng of GABA. The detection limit for GABA in a diluted standard solution was 20 pg per injection (15 μl), but the detection limit for GABA in a diluted tissue sample solution was Ca. 50 pg per injection (15 μl).

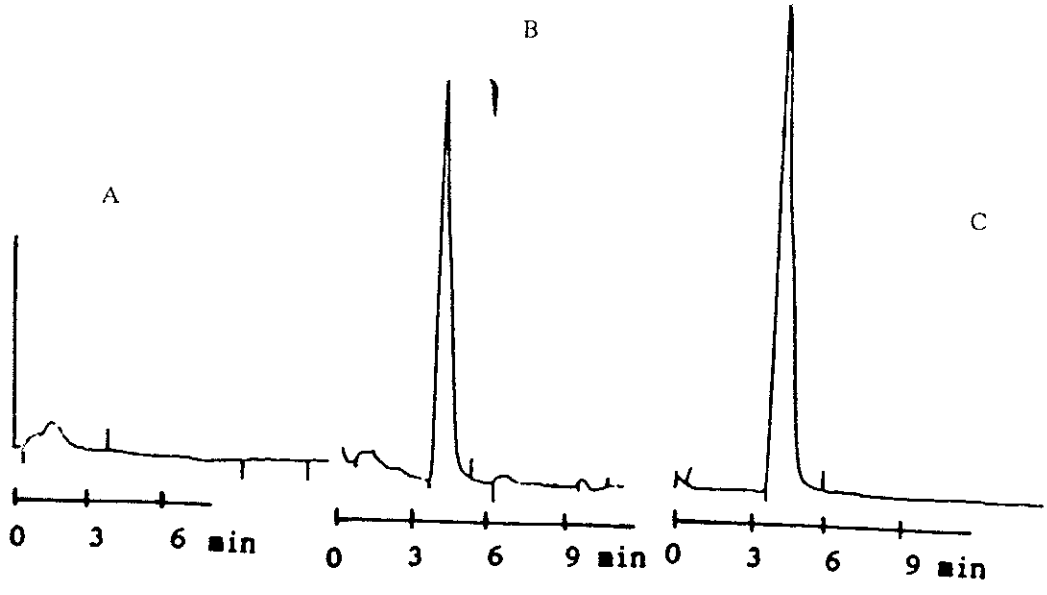

Fig. 1. Choromatogram of OPA-BME derivatives
(A) Blank reagent; (B) standard of GABA; (C) rat brain

DISCUSSION

Various methods have been developed (10-12) for the quantification of GABA. Recently dansyl-GABA (13) has been measured by HPLC. In the present study, the derivative is actually formed during the tissue extraction system, hence there is no complicated handling procedure and this is one of the sevesral advantages over other methods. The run time is quite short (less than 5 min) and sensitivity (less than 1 pmol) is satisfactory. The method has adequate precision coefficient.

The present method, which is more rapid and more sensitive than other previous methods described by others (14), is suitable for the routine assay of brain GABA and for studying the regulatory interaction between GABA and drugs in the brain, since it is known that the reproducibility and sensitivity of the method had been excellent. Measurement of GABA levels in brain may be helpful for diagnosis and therapy of brain disorders (15, 16, 17, 18). The present method provides a rapid sensitive, selective and relatively inexpensive assay for this purpose.

REFERENCES

1. Okada Y. Distribution of gamma-aminobutyric acid (GABA) in the layers of superior colliculus of the rabbit. Brain Res. 75: 362 - 365; 1974.

2. Jung M.J., Lippert B., and Metcalf B.W. The effect of 4-aminohex-5-ynoic acid (gamma - ace - tylenic GABA, gamma-ethynyl GABA), a catalytic inhibitor of GABAtransaminase on brain metabolism in vivo. J. Neurochem. 28, 717 - 723; 1977.

3. Norman E.J., Wee E.L., and Berry H.K. GABA depletion and behavioral changes produced by intra-ventricular putrescine in chicks. J. Chromatography. 337: 21 - 29; 1985.

4. Enna S.J. and Synder S.H. Regulatory role of gamma-aminobutyric acid in pituitary hormone secretion. J. Neurochemistry. 26: 221 - 228; 1976.

5. Baxter C. F and Roberts E. The gamma- aminobutyric acid-a-ketoglutaric acid transaminase of beef brain. Methods of Neurochem. 3: 1135 - 1139; 1972.

6. Seiler N. Polyamine metabolism and function in brain. Neurochem. Res. 3: 95 - 110; 1981.

7. Lindgren S. and Anden N.E. Effect of the normal nerve impules flow on the synthesis and utilization of GABA in the rat substantia nigra. J. Neural. Transmiss. 61: 21 - 34; 1985.

8. Guy E., Griesmann, Wal-yee chan and Owen M. Rennert: Determination of gamma- aminobutyric acid by reversed-phase high-performance liquid chromatography and pre-column labeling for fluorescence detection. J. chromatography. 230: 121 - 124; 1982.

9. Albers R.W. and Brady R.O. The distribution of glutamic decarboxylase in the nervous system of the monkey. J. Biological. Chemistry. 234: 926 - 928; 1959.

10. Herranz A.S., Lerma J. and Martin del Rio. R. Determination of gamma - aminobutyric acid in physiological samples by a simple rapid high-performance liquid chromatographic method. J. Chromatography. 309: 139 - 144; 1984.

11. Loscher W. GABA in plasma and cerebrospinal fluid of different species. J. Neurochemi-stry. 32: 1587 - 1591; 1979.

12. Martin del Rio R. Gamma - Aminobutyric acid system in rat oviduct. J. Biol. chem. 256: 9816 - 9819; 1987.

13. Saller C. and Czupryna M.J. Gamma- aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives. J. Chromatography. 487: 167 - 172; 1989.

14. Okano y., Kadota T. and Nagata J. Quantification by selected ion monitoring of pipecolic acid, proline, gamma - aminobutyric acid and glycine in rat brain. J. Chromatography. 310: 251 - 259; 1984.

15. Loscher W., Bohme G. and Schafer H. Effect of metabolites of valproic acid on the metabolism of GABA in brain and brain nerve endings. Neuropharmacol. 20: 1187 - 1192; 1981.

16. Kannenberg K., and Martin H. Groschup and Cellular prion protein and GABA receptors. Neuroreport 7: 77 - 80; 1995.

17. Petty F., Kramer GL. and Davis LL., Plasma gamma-aminobutyric acid (GABA) predicts outcome in patients with alcohol dependence. Prog. Neuropsychopharmacol. Biol. psychiatry. 21(5): 809 - 16; 1997.

18. HaJ H., Knauer S. and Moody E. Direct enhancement of GABA-ergic neurotransmission by ammonia. Adv. Exp. Med. Biol.420: 85 - 94; 1997.