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Note

Determination of some Amino Acids in the Central Nervous System by Thin-layer Chromatography

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Free amino acids (glutamic acid, γ-amino-butyric acid and glycine) from the central nervous system of albino mice have been indentified and determined quantitatively. This method is convenient for determination of certain amino acids in small samples of nervous tissue (50—1000 mg).

The procedure is simple, economical and rapid. Separation on the chromatogram is good and reproducible, due to a specific saturation of the chromatographic-tank atmosphere.

Previous studies have indicated that γ-amino butyric acid (GABA), glycine and taurine may act as inhibitory neurotransmitters, whereas glutamic and aspartic acid may act as excitatory neurotransmitters in the central nervous system (CNS) of vertebrata[1–4].

There is a number of methods for the separation and determination of free amino acids in CNS: thin-layer chromatography (TLC)[5,6], paper chromatography (PC)[7], and gas-liquid chromatography (GLC)[8,9]. The most precise method is the ion-exchange chromatography by means of automatic amino acid analyser (AAA)[10–11]. TLC on silica-gel or on cellulose layers is very convenient for analysis of free amino acids in nervous tissue[12]. A number of TLC solvent systems, especially the one-dimensional ones, have a shortcoming: poor separation of glycine and serine. The purpose of this work was to obtain good separation of serine and glycine, as well as other free amino acids in the mouse brain.

EXPERIMENTAL

Materials and Methods

Male albino mice weighing 17—23 g, were used in all experiments.

The absorbance values were measured with a Beckman DU UV/VIS spectrophotometer.

Standard samples of amino acids from F. Hoffman-La Roche and Co., Basel, Switzerland, were used.

Ethanol abs., methanol, ninhydrine and cadmium acetate were obtained from Merck, Darmstadt, Germany.

n-Butanol and glacial acetic acid were products of »Laphoma«, Skopje, while ammonia 25% was a product of »Azot«, Goražde, Yugoslavia.

Tissue Preparation and Extraction

The animals were decapited, the brains were very quickly weighed and placed in previously refrigerated glass-homogeniser, containing 85% ethanol (1 : 8 g/v). The

tissue was homogenized for 90 sec at 0 °C and centrifuged at 38 000 g and —10 °C for 30 minutes. The supernatant was decanted and stored evernight at —20 °C to precipitate the lipids. The lipids were separated by centrifugation at —10 °C and 18 000 g for 10 minutes.

The supernatant (0.3 ml) was used directly for TLC.

Thin-layer Chromatography

Commercially available plates (20 × 20 cm) with 0.25 mm thick Silica-gel F_{25} layer (Merck, Darmstadt) were used throughout the experiments to control the chromatographic effect in UV-light (254 nm).

The solvent system ethanol-NH_4OH (25%) in ratio 100 : 28 was used for development. The solvent was allowed to ascend 15 cm with the walls of chromatographic tank lined with two filter-paper pieces to saturate the atmosphere. As a supplementary saturation a 10 ml glass vessel containing 25% NH_4OH was placed in the tank.

The development of the chromatogram took about 2 hours and was performed twice at room temperature. After the first development the chromatogram was dried at 100 °C for 30 minutes. After the second development with identical solvent system, the minimum period of drying was two hours at 105 °C, for the removal of even traces of ammonia.

The chromatograms were stained with ninhydrin-butanol reagent following the procedure of Barrollier[13]. The coloured spots of the amino acids were marked and removed quantitatively by covering them with a 15% solution of collodion in acetone. The coloured spots were placed into centrifugation tubes and to each 3.5 ml of a 0.5% $Cd(CH_3COO)_2$ solution and 0.1% glacial acetic acid were added. The test tubes were put in dark place for one hour and shaken periodically. They were centrifuged at 9 500 g for 15 min. The supernatant was decanted into glass cells and absorbance measured at 520 nm.

Standard samples of amino acids (0.1 μM/0.1 ml) were prepared and amino acid recovery was determined by addition of the relevant amino acid (5 μM/0.5 g of tissue) to brain tissue extracted previosly 5—6 times with an excess of 95% ethanol. Such samples were subjected to the extraction process.

Rechromatography by the method of Fahmy *et al.*[14] has shown that the separation of these three amino acids was good. It was shown that using this method as small quantity of tissue as 50 mg was sufficient to perform the experiment.

RESULTS AND DISCUSSION

TL chromatogram of a mixture of glutamic acid, GABA and glycine is shown in Figure 1. The R_f values of pure standards of a series of amino acids in chromatographic system used, with each value given as the mean of five measurements and accompanied by standard error, are given in Table I. Recovery values for glutamic acid, GABA and glycine were found to be 92%, 93.5% and 90.5% respectively, and their concentrations as determined in albino mouse brain were compared with results obtained by Agrawal et *al.*[15] (Table II).

The primary purpose of our work was to obtain a good separation of serine and glycine, and this problem was solved satisfactorily. In addition to this problem, it was possible to obtain a good separation of many other amino acids from the albino mouse brain (Figure 2.).

However, even if a good resolution of these amino acids was obtained, alanine and threonine were still so close together on the chromatogram, that this method for their quantitative analysis should be avoided.

It should be stressed that the direct use of the supernatant without previous evaporation gives better results for determination of free amino acids. Evaporation to dryness and dissolution of the dry residue was found to yield 10—15% lower recovery.

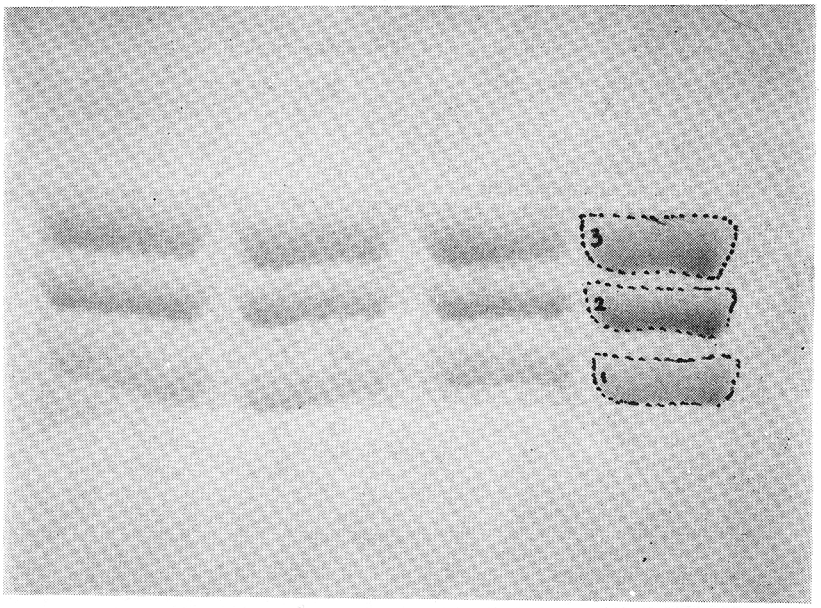

Figure 1. Thin-layer chromatographic separation of 1. Glutamic acid, 2. GABA and 3. Glycine

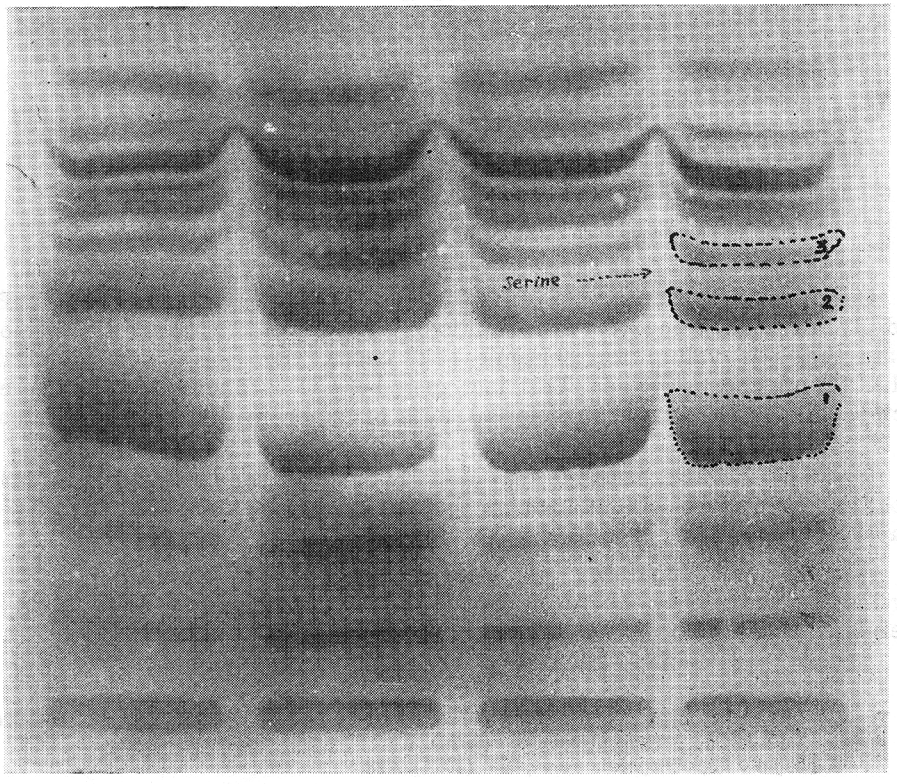

Figure 2. Thin-layer chromatographic separation of amino acids from albino mouse brain

TABLE I

R_f-values of amino acids in chromatographic system used

	$R_f \pm$ S.E.M.
1. Glutamine	0
2. Phosphoethanol amine	0
3. Ethanol amine	3 ± 0.1
4. Arginine	10 ± 0.2
5. Aspartic acid	17 ± 0.2
6. Lysine	21 ± 0.2
7. Glutamic acid	28 ± 0.3
8. GABA	38 ± 0.2
9. Serine	42 ± 0.1
10. Glycine	45 ± 0.2
11. Alanine	52 ± 0.3
12. Threonine	56 ± 0.1
13. Tyrosine	61 ± 0.2
14. Taurine	62 ± 0.2
15. Valine	70 ± 0.3
16. Urea	70 ± 0.2
17. Leucine	75 ± 0.1
18. Iso-Leucine	75 ± 0.2
19. Phenylalanine	77 ± 0.2

TABLE II

Amino acid level in albino mouse brain

Amino acid	Recovery %	Our results μM/g	Agrawal et *al.*'s results μM/g
Glutamic acid	92.0	9.36 ± 1.09	11.50
GABA	93.5	2.04 ± 0.13	2.52
Glycine	90.5	1.96 ± 0.15	1.61

Our results concerning the examined levels of the glutamic acid, GABA and glycine when compared with results of Agrawal et *al.*[15] (Table II.), demonstrate that our values for glutamic acid and GABA are lower, while the value for glycine is higher. This fact may be due to a number of different factors, of which the use of different sorts of experimental animals need not be the least.

It should be also stressed that a permanent saturation with NH_3 gives a better resolution of amino acids on the chromatogram, compared with filter-paper saturation.

A satisfactory separation of aspartic acid, glutamic acid, GABA, serine, glycine and taurine by means of the method described in this work might be useful in small laboratories without expensive equipment.

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SAŽETAK

Određivanje nekih aminokiselina u centralnom nervnom sistemu tankoslojnom hromatografijom

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Slobodne aminokiseline (glutaminska kiselina, γ-aminomaslačna kiselina i glicin) iz centralnog nervnog sistema albino-miša identifikovane su i kvantitativno određivane. Ta je metoda pogodna za određivanje tih aminokiselina u malim uzorcima nervnog tkiva (50—1000 mg).

Postupak je jednostavan, ekonomičan i brz, a osim toga pruža mogućnost analize i nekih drugih aminokiselina iz centralnog nervnog sistema. Razdvajanje na hromatogramu je dobro i reproducibilno zbog drugačijeg zasićenja atmosfere hromatografskog tanka.

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