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# GABA-T in Bovine Medulla Cells: Kinetic Properties and Comparison with GABA-T from Other Tissues

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4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T) has been found in adrenal medulla. The enzyme from this tissue is very similar to those found in other tissues in respect to their mitochondrial localization, optima pH and responses to cofactor. The enzyme from medulla has substrate km values similar to those for the brain enzyme, while it differs from those found for other tissues such as kidney, liver and platelets.

4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T EC. 2.6.19) the enzyme principally responsible for the catabolism of 4-aminobutyrate (GABA) in the CNS, is now known to occur in some peripheral organs including liver, kidney and platelets. (5,9-12) and we report here the presence of this enzyme in bovine adrenal medulla cells. The present study was undertaken to define the properties of GABA-T isolated from adrenal medulla and to compare properties of this enzyme with those of brain and other tissues.

## Materials and Methods

Bovine adrenal glands were supplied by the local slaughter house immediately placed in cold 0.32 M sucrose and dissected in the hour following the death of the animals.

*Subcellular fractionation.* The method for subcellular fractionation was based on that of AUNIS *et al.* (1). The adrenal glands were deffated and, after fine dissection, the medulla were suspended in 10 volumes (w/v) of 0.32 M sucrose containing 0.1 mM EDTA and 1 mM dithiotreitol (AET). The mixture was homogenized in a mechanical Potter Elvehjem with teflon pestle. This homogenate was centrifuged for 10 min at 800 *x g*. The supernatant was passed through a cheese-cloth in order to remove floating fat, this fraction was considered as homogenate which was centrifuged at

11.000 *x g* for 20 minutes. The supernatant was considered as «soluble fraction». The pellet was then suspended in the homogenization medium and the suspension was layered on 1.6 M sucrose into Swing-out tubes and centrifuged for 90 minutes at 100.000 *x g*. Two bands were obtained, one, on the top with 1.6 M sucrose, referred as purified mitochondria and other at the bottom, referred as «chromaffin granules».

*Enzyme extraction.* Purified mitochondria and chromaffin granules were separated and each fraction resuspended in the homogenization medium and centrifuged for 20 minutes at 15.000 *x g*. Each pellet was homogenized in Potter-Elvehjem with glound glass pestle, in the presence of 5 mM potassium phosphate buffer pH 7.5; 1 mM AET; 0.1 mM EDTA-$Na^+$; 0.04 mM PLP and 0.3% Triton-X-100. These homogenates, referred to as «pure mitochondria» (PM) and chromaffin granules (CG) were used as enzymatic preparation. All fractions were dialyzed overnight against extraction mixture.

*GABA-T activity.* The enzyme activity was assayed by both radiometric and colorimetric methods. The latter was developed in our laboratory and the technique is described below.

*Radiometric method.* It was assayed according to BAXTER (2) except that the total volume was 0.1 ml.

*Colorimetric method.* The incubation mixture contained: 150 mM pyrophosphate buffer pH 8.6; 0.04 mM reduced glutathione 0.1 mM PLP; 12.5 mM 2-oxoglutarate; 31 mM GABA and enzymatic extract. Incubations were carried out for 1 h a 37° C. The reaction was stopped by addition of 300 $\mu$l of 10% (w/v) trichloroacetic acid and centrifuged for 10 minutes in clinical centrifuge. Blanks without 2-oxoglutarate and GABA were run; they were added after stopping the reaction. The succinic semialdehyde formed in the aminotransferase reaction was determined in the supernatant by the hydrazone method (4).

Proteins were measured according to LOWRY *et al* (6).

*Km determinations.* They were performed by the colorimetric method using pyrophosphate as buffer and substrates concentrations were varied between 0.08-0.25 mM for 2-oxoglutarate and 0.1-1 mM for GABA. The results are means of, at least, three determinations.

## Results

*Enzyme activity.* The GABA-T activity of adrenal medulla was a lineal function of both incubation time and extract protein concentration (Figure 1a, 1b).

*Subcellular localization.* The purity of subcellular fractions was checked by marking enzymes: LDH, for soluble fraction; fumarase, for mitochondrial fraction and dopamine-$\beta$-hydroxy-lase for chromaffin granules. GABA-T from adrenal medulla is localized, as in brain, in mitochondrial fraction. In crude mitochondria the GABA-T recuparation was of a 72% and after separating the chromaffin granules from this fraction the enzyme was found in pure mitochondria. The 9% of activity found in chromaffin granules can be attributed to mitochondria contamination according to the results obtained by marking enzymes (Table I). In the obtention of pure mitochondria the enzyme was purified 8 times over the homogenate.

*Enzyme properties.* The activity of adrenal medulla GABA-T was about 25%

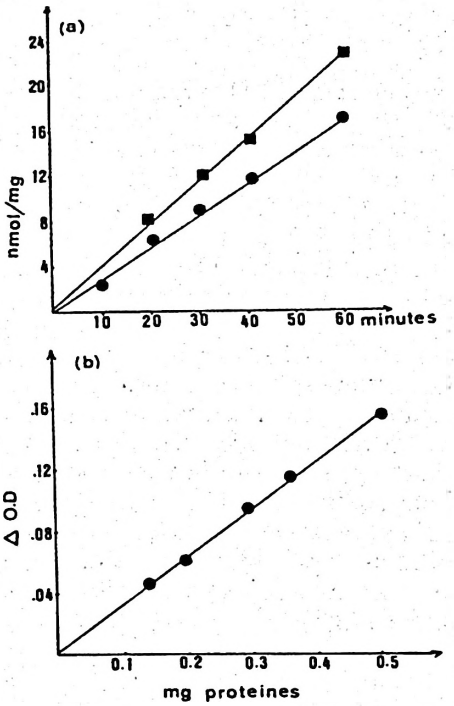


Fig. 1. *Effect of incubation time and protein concentration on GABA-T from adrenal medulla.* (a) Effect of incubation time: ■—■—■ plus PLP; ●—●—● without PLP. (b) Effect of protein concentration. Crude mitochondria and the colorimetric method were used in these determinations.

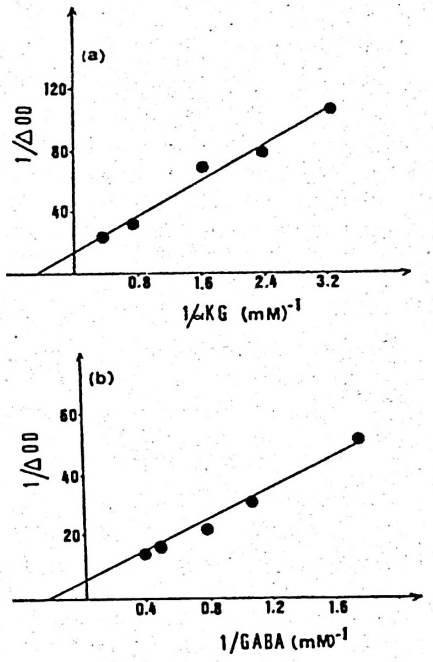


Fig. 2. *Double reciprocal plots of the effect of varying GABA (a) or 2-oxoglutarate (b) concentration on GABA-T by using the colorimetric method.*

lower in the absence of added cofactor (PLP) than in its presence (Figure 1a, Table II). This seems to indicate that the requirement of this enzyme for its cofactor is lower than that of the brain enzyme (12) or the enzyme from other sources as kidney, liver and platelets (11), where the activity in the absence of PLP is about 60% lower than it its presence. The GABA-T from adrenal medulla, as in brain, shows higher activity whit pyrophosphate buffer than with Tris-HCl (8). The optimal pH of the enzyme from medulla was similar to that of brain enzyme (Figure 3, Table II).

Table I. *Subcellular distribution of GABA-T from bovine adrenal medulla.* Activities were assayed by the radiometric method.

| Fraction | Total activity (nmol/m) | Yield % Activity | Specific activity (nmol/m/mg) | Purification |
|---|---|---|---|---|
| Homogenate | 555.6 ± 21.0 | 100 | 0.4 ± 0.015 | 1 |
| Supernatant | 12.5 ± 2.5 | 2 | 0.03 ± 0.006 | — |
| Crude mitochondria | 403.1 ± 34.7 | 72 | 1.1 ± 0.09 | 3 |
| Purified mitochondria | 328.0 ± 4.1 | 59 | 3.3 ± 0.04 | 1 |
| Chromaffin granules | 50.2 ± 4.8 | 9 | 0.3 ± 0.03 | — |

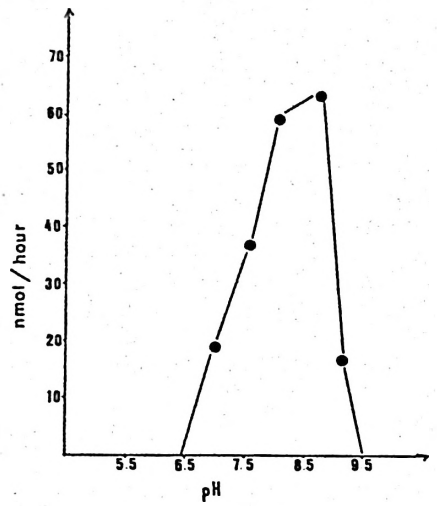


Fig. 3. *Activity of GABA-T as a function of pH.* Phosphate buffer (pH 6.5-7.5); Tris-HCl buffer (pH 8-9.5) were used to obtain the desired pH.

The Km for GABA and 2-oxoglutarate, measured by Lineweaver-Burk plots, were 6.9 ± 0.9 and 2.5 ± 0.29 respectively (Figure 2a, 2b. Table II). These values represent a lower affinity by the substrates than that found by kidney, liver or platelets (12) and they are of the same order than those from brain (7) although the Km for both substrates found in brain presents a great variability depending on the brain source (11).

Table II. *Properties of GABA-T from adrenal medulla.*

| Parameters | Values |
|---|---|
| Optime pH | 8.6 |
| Km (mM): | |
| GABA | 6.9 ± 0.9 |
| 2-oxoglutarate | 2.5 ± 0.3 |
| % Activity with: | |
| + PLP | 100 |
| — PLP | 73 |
| Tris-HCl buffer | 100 |
| Pyrophosphate buffer | 265 |

## Discussion

The adrenal GABA-T enzyme resembles to the brain enzyme in its mitochondria localization, Km values for both substrates, optimal pH and responses to cofactor and buffers. Although the presence of GABA-T in adrenal medulla is not surprising since in this tissue GAD type I and II has been found (3), however there is not any answer to explain the meaning of GABA by-pass in adrenal medulla, although one can assume several hypotheses: 1) since adrenal cells are of neural origin, ectodermic origin, as the brain, the presence of the GABA by-pass in them could be implicated in the regulation of synthesis and/or release of adrenal catecholamines. All these theories deserves further studies which are known in an undertaking.

## Resumen

Se demuestra la existencia en médula adrenal bovina de la 4-aminobutirato-2-oxoglutarato aminotransferasa (GABA-T). La enzima de esta procedencia es muy semejante a las encontradas en tejidos como cerebro, hígado, riñón y otros, en lo que se refiere a su localización mitocondrial, pH óptimo y requerimientos del piridoxal fosfato. Sin embargo, el estudio de la afinidad por sus sustratos, GABA y 2-oxoglutarato, realizado con enzima medular permite comprobar que presenta un comportamiento más semejante al de la enzima cerebral que al de las enzimas procedentes de hígado, riñón y plaquetas sanguíneas.

## References

1. AUNIS, D., ALLARD, D., MIRAS PORTUGAL, M. T. and MANDEL, P.: *Biochem. Biophys. Acta*, 393, 284-295, 1975.
2. BAXTER, C. F.: In «Methods in Neurochemistry» (Fried, R., ed.) Vol. 3 Marcel Dekker, New York 1972, pp. 3-73.
3. FERNÁNDEZ-RAMIL, J. M., SÁNCHEZ-PRIETO, J. and GONZÁLEZ, M. P.: *Rev. esp. Fisiol.*, 38, 91-96, 1982.

4. GONZÁLEZ, M. P., CAÑADAS, S., SÁNCHEZ-PRIETO, J. and MORALES, V.: *Rev. esp. Fisiol.*, 39, 179-182, 1983.
5. LANCASTER, G., MOHYUDDIN, F., SERIVNER, C. R. and WHELAN, D. T.: *Biochem. Biophys. Acta.*, 297, 229-240, 1973.
6. LOWRY, O. H., ROSENBROUGH, N. R., FARR, A. and RANDALL, R. J.: *J. Biol. Chem.*, 193, 265-275, 1951.
7. NGO T. T. and TUNNICLIFF, G.: *Com. Biochem. Physiol.*, 59C, 101-104, 1978.
8. VAN KEMPER, G. M. J., VAN DEN BERG, C. J., VAN DER HELM, H. J. and VELDTRA, H.: *J. Neurochem.*, 12, 581-588, 1965.
9. VASILEV, V. Y., EREMIN, V. P., SEVERIN, E. S. and SYTINSKLL, L. A.: *Biokimiya*, 38, 355-364, 1973.
10. WHITE, H. L,: *Science*, 205, 696-698, 1979.
11. WHITE, H. L. and FAISON, L. D.: *Brain Res. Bull.*, 5, 115-119, 1980.
12. WHITE, H. L. and SATO, T. L.: *J. Neurochem.*, 31, 41-47, 1978.