Title

Ornithine decarboxylase activity associated with a particulate fraction of brain

Journal

Neurochemical Research, 11(12)

ISSN

0364-3190

Author

Bondy, SC

Publication Date

1986-12-01

DOI

10.1007/bf00967743

Neurochemical Research, Vol. 11, No. 12, 1986, pp. 1653–1662

ORNITHINE DECARBOXYLASE ACTIVITY ASSOCIATED WITH A PARTICULATE FRACTION OF BRAIN

S. C. BONDY
Department of Community and Environmental Medicine
University of California, Irvine
Southern Occupational Health Center
19722 MacArthur Boulevard
Irvine, California 92715

Accepted May 1, 1986

The enzymic decarboxylation of ornithine by adult rat brain largely occurs in the particulate fraction. The activity is primarily due to ornithine decarboxylase (ODC) as evidenced by several criteria: i) the concurrent production of equimolar amounts of CO_2 and putrescine, ii) the sensitivity of the reaction to difluoromethylornithine (DFMO), a specific inhibitor of ODC, iii) the lack of major effect of two inhibitors of ornithine-2-oxo-acid transaminase, upon the DFMO-sensitive component of decarboxylation, iv) the failure to profoundly reduce decarboxylation activity in the presence of a large excess of many aminoacids which could compete for non-specific decarboxylases. The insoluble ODC activity appears largely within synaptosomal and mitochondrial-enriched morphological fractions, yet cannot be attributed to trapped soluble ODC. Particulate ODC has a pH optimum and kinetic parameters that differ from those of soluble cerebral ODC.

INTRODUCTION

Ornithine decarboxylase (ODC, EC 4.1.1.17) is a soluble enzyme bringing about the conversion of ornithine to putrescine. This enzyme is at high levels in proliferating and developing tissues (1) and in tissues following endocrine activation (2, 3). The mature nervous system has very low levels of ODC (4, 5). This enzyme has been reported to be present in synaptosomal fractions (6) but this finding has been questioned (7). The successful identification of this enzyme is complicated by the existence of non-specific amino acid decarboxylases and a variety of transaminases

0364-3190/86/1200-1653$0.500/0

in brain tissue (7). These enzymes could cause the production of $^{14}CO_2$ from carboxyl-labeled ornithine without involving ODC. However, if the rate of production of labeled putrescine from generally tritiated ornithine is assayed, this difficulty can be circumvented. Another useful criterion is the use of the specific inhibitor of ODC, difluoromethyl ornithine (DFMO) (8). Thus, the non-specific decarboxylation of ornithine by rat liver nuclei has been reported to be completely insensitive to 0.4×10^{-3} M DFMO and to result in the production of no putrescine (9).

This work describes an insoluble form of ornithine decarboxylase that is present in brain particulate fractions. By use of various inhibitors and determination of [^{3}H]ornithine conversion to putrescine, other possible mechanisms of ornithine catabolism are substantially eliminated.

EXPERIMENTAL PROCEDURE

Preparation of Morphological Fractions. A crude particulate fraction was prepared by centrifugation (40,000 *g* × 10 min) of a 5% (w/v) homogenate of adult rat tissue in 0.04 M Tris-HCl pH 7.4. The precipitate was resuspended in tris, to the original homogenate volume.

A nuclear fraction was prepared by tissue homogenization on 0.32 M sucrose – 0.04 M Tris-pH 7.4. This homogenate was vigorously mixed with 2.47 volumes of 2.4 M sucrose – 0.04 M Tris pH 7.4 to give a final sucrose concentration of 1.8 M. This suspension was centrifuged (40,000 *g* 30 min) and the resulting pellet largely consisted of nuclei as judged by phase contrast microscopy and the RNA/DNA ratio (10).

A partial separation of intact synaptosomes and mitochondria was performed using the method of Gray and Whittaker (11) as modified by Dodd et al. (12). A 5% homogenate of brain in 0.32 M sucrose was centrifuged (1,000 *g* × 10 min). After removal of intact cells, nuclei and tissue fragments by this low speed centrifugation, the supernatant was layered over 1.2 M sucrose and centrifuged at 180,000 *g* for 15 minutes. The pellet obtained at the bottom of the tube was taken as the mitochondrial fraction. The material at the interface between the 0.32 and 1.2 M sucrose was diluted with four volumes 0.32 M sucrose and layered over 0.8 M sucrose. After recentrifugation at high speed, exactly as for the previous gradient, the pellet was taken as the synaptosomal fraction while the interface material, over the 0.8 M sucrose, constituted the myelin fraction. All pellets were taken up in 0.04 M Tris, pH 7.4 prior to enzyme assay. This method produces fractions comparable to the original procedure of Gray and Whittaker (11) in a much shorter period. This is important when assaying a relatively unstable enzyme such as ODC.

Ornithine Decarboxylase Assay. ODC was assayed by measurement of evolved $^{14}CO_2$ from carboxyl-[^{14}C]ornithine (55.9 mCi/mmol, New England Nuclear, Boston, MA) (4). 0.7–0.9 ml of various tissue preparations was added to 50 μl pyridoxal phosphate solution (1 mM) and 50 μl [^{14}C]ornithine in 0.045 M dithiothreitol and 0.01 M Tris-HCl, pH 7.2. The final ornithine concentration was 2.5 μM.

Incubation (37°C, 30 min) was carried out in a sealed tube and was terminated by injection of 1 ml 2 M acetic acid into the reaction mixture. Evolved $^{14}CO_2$ was trapped on a paper wick containing hyamine suspended above the reaction mixture. The decarboxylation process was linear for up to 1.5 hr under these conditions. Decarboxylation, not attributable to ODC, was determined by running a parallel incubation in the presence of 5 mM difluoromethyl ornithine, a specific inhibitor of ODC (8).

ODC was also assayed by chromatographic separation of [^{3}H]putrescine synthesized from [^{3}H]ornithine after an incubation similar to that described above (13). L-[2,3,^{3}H]ornithine (17 Ci/mol) was substituted for [^{14}C]ornithine and at the end of incubation, reaction mixtures were directly transferred to a strong cation exchange paper (Whatman cellulose phosphate, P81) and subjected to descending chromatography in 0.1 N NH_4OH. Under these conditions labeled ornithine migrated with an R_f of 0.6 while putrescine remained at the origin, where the spot could be cut out and assayed for radioactivity. The location of putrescine was confirmed using aliquots of [2,3-^{3}H(N)]putrescine dihydrochloride (19 Ci/mol).

RESULTS

The soluble fraction of kidney from adult rat had a very high ODC activity which was virtually totally blocked in the presence of 5 mM DFMO; corresponding fractions of brain and liver contained very little ODC (Table I). The particulate fraction from all three tissues had a significant capacity for effecting $^{14}CO_2$ liberation from carboxyl-labeled [^{14}C]ornithine and 5 mM DFMO inhibited this process by 43–67% (Table I). Since DFMO is believed to be a highly selective inhibitor of ODC, this implied the existence of particulate ODC, and this possibility was further studied in the brain.

The fractionation of cerebral homogenates into particulate and soluble components was investigated after freezing the homogenate to −20°C and then thawing in order to disrupt organelles (Figure 1). This treatment caused a reduction of decarboxylation activity in both particulate and soluble fractions but the distribution of activity between fractions remained the same. The freezing procedure did not appear to solubilize the particulate decarboxylation activity. The lower total activity of the original homogenate suggested an inhibitory effect of the soluble fraction upon

TABLE I

ORNITHINE DECARBOXYLASE IN SOLUBLE AND PARTICULATE FRACTIONS OF BRAIN, KIDNEY, AND LIVER

		−DFMO	+DFMO	DFMO-Sensitive activity
Brain	Soluble	46 ± 5	30 ± 9	16
	Particulate	1725 ± 41	570 ± 87	1155
Kidney	Soluble	5825 ± 472	39 ± 3	5786
	Particulate	5700 ± 613	3241 ± 85	2459
Liver	Soluble	21 ± 2	10 ± 1	11
	Particulate	5109 ± 206	2046 ± 632	3063

Data are expressed as pmol CO_2 evolved/g tissue per hr.

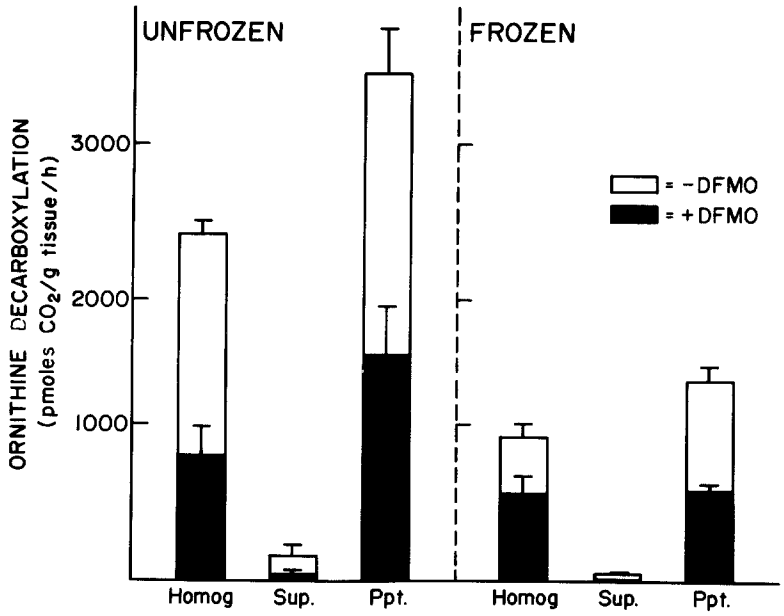

FIG. 1. Decarboxylation of ornithine by soluble and particulate fractions prepared from fresh or frozen brain homogenates. DFMO, where present, was at 5 mM. Bars indicate standard error of the mean.

particulate decarboxylation activity. However, when remixing of separated particulate and soluble fractions was tested, no inhibitory interaction was found. Reconstitution of the unfrozen particulate and soluble fractions containing 91–94% and 6–9% of total DFMO-sensitive decarboxylation activities respectively, resulted in a total enzyme activity that was 103 ± 4% of the expected total. The lower activity of the original homogenate relative to the particulate fraction may have reflected greater proteolytic opportunities in the crude homogenate. In view of this and since the soluble activity was very low, several of the following studies utilized whole homogenate of brain.

In order to minimize sources of decarboxylative activity other than ODC, incubation of brain homogenates was carried out in the presence of an excess of many amino acids which could saturate any non-specific amino acid decarboxylation activity. $^{14}CO_2$ production was assayed in the presence of 1.6×10^{-4} M of each of the following l-amino acids: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine methionine, isoleucine, leucine, tyrosine, phenylalanine and 0.8×10^{-4} M cystine. Both DFMO-sensitive and -resistant

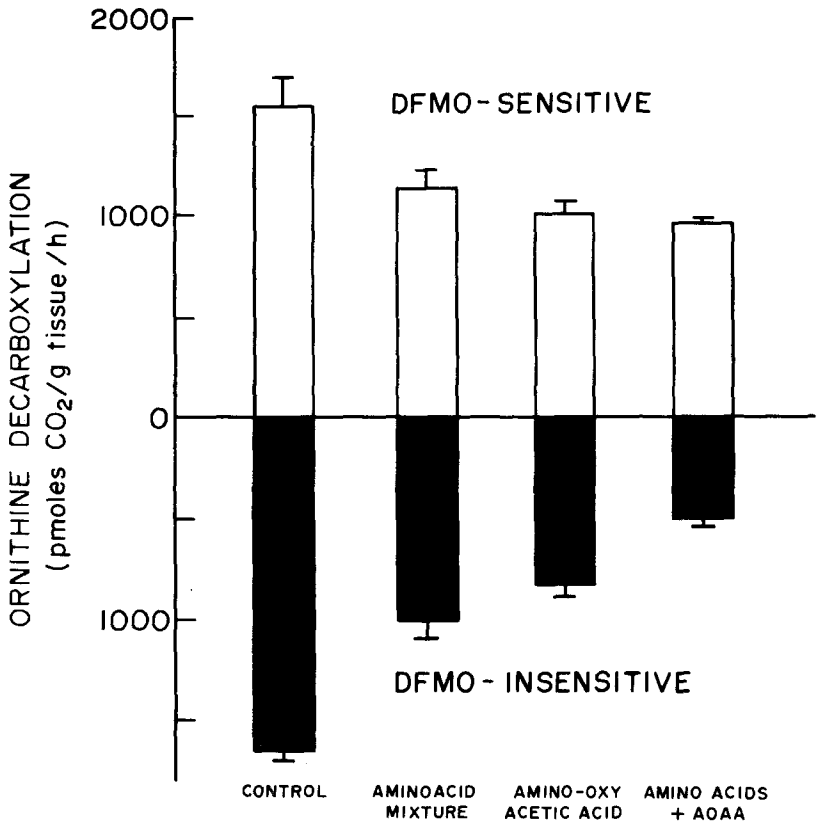

FIG. 2. Decarboxylation of ornithine by brain homogenate in the presence of an amino acid mixture containing 1.6×10^{-4} M of several amino acids (see text), or 5×10^{-6} M aminooxyacetic acid. Shaded columns show DFMO-insensitive activity and open columns represent activity inhibited by 5 mM DFMO.

components of decarboxylation were reduced by 29 and 39%, respectively (Figure 2).

Incubation of cerebral homogenates was also performed in the presence of 5×10^{-6} M aminooxyacetic acid (AOAA). AOAA is an inhibitor of transaminases and at the concentration used, ornithine-2-oxo acid aminotransferase (EC 2.6.1.13) is virtually completely inhibited (14). The latter enzyme is on a pathway whereby the carboxyl group of ornithine is transformed by transamination to glutamic semialdehyde and thence to glutamate (15). Following this, a variety of transformations leading to CO_2 liberation are possible either by direct decarboxylation or transa-

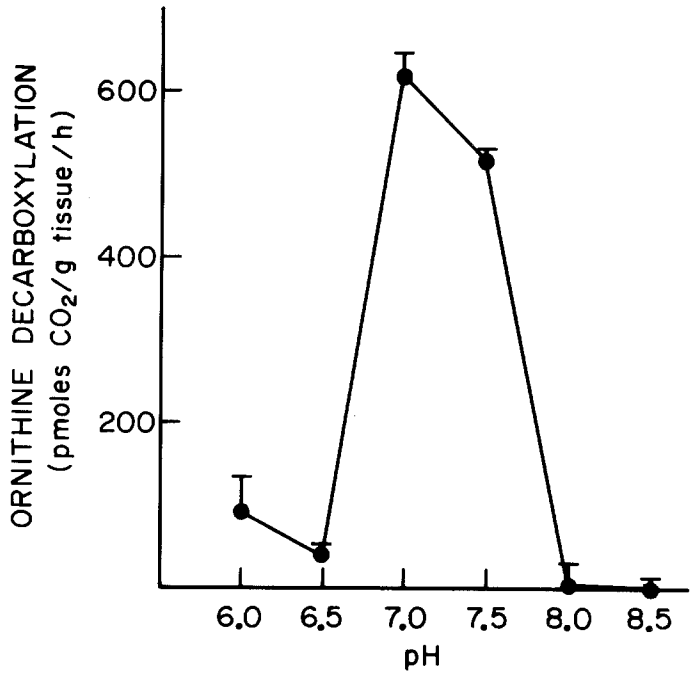

FIG. 3. Effect of varying pH on decarboxylation of ornithine by cerebral particulate fraction. Bars represent standard errors of the mean.

mination and entry of 2-oxoglutarate into the tricarboxylic acid cycle. AOAA (5×10^{-6} M) inhibited both DFMO-sensitive decarboxylation activity (by 35%) and DFMO-resistant activity (by 50%, Figure 2).

The concurrent presence of the amino acid mixture and AOAA caused a loss of decarboxylation of 38% for the DFMO-sensitive process and 70% for the DFMO-resistant component. In the case of the DFMO resistant activity, the combined effect of decarboxylation and transamination inhibitors (70% inhibition) approached the expected additive value of 89%. However, the DFMO sensitive decarboxylation was only a little more inhibited by the simultaneous presence of both classes of inhibitor (38%) than by either alone (29 and 35%).

The use of a more specific transaminase inhibitor (amino-4-hex-5-ynoic acid), acetylenic GABA (courtesy of Merrell Dow Research Institute, Strasbourg, France) at a concentration of 10^{-3} M inhibited decarboxylation activity in a manner parallel to that of AOAA (data not shown). At this concentration acetylenic GABA inhibits ornithine-2-oxo-acid transaminase in addition to GABA transaminase (15).

TABLE II
ORNITHINE DECARBOXYLASE ACTIVITY IN PARTICULATE SUBFRACTIONS OF RAT BRAIN HOMOGENATE

Fraction	ODC (pmol CO_2 evolved/mg protein per hr)
Nuclear	10.0 ± 0.1
Mitochondrial	34.4 ± 0.4
Synaptosomal	51.3 ± 8.5
Myelin	0.5 ± 0.1

Data represent CO_2 evolution that is inhibited in the presence of 5 mM DFMO.

When ODC activity was measured by chromatographic assay of [^{3}H]putrescine formation from [^{3}H]ornithine, similar activity levels of ODC were found for brain homogenates, as the corresponding results obtained by measurement of $^{14}CO_2$ from carboxyl-labeled ornithine. Ornithine decarboxylase activity in brain homogenate was 22 ± 4% of that observed in the kidney soluble fraction (5.4 ± 0.8 nmol CO_2/g tissue per hr) as measured by the rate of CO_2 evolution. The corresponding ornithine decarboxylase value of brain homogenate when [^{3}H]putrescine formation was measured, was 20 ± 5.6%.

The DFMO sensitive decarboxylation of [^{14}C]ornithine by brain homogenates had a pH optimum of 7.0–7.5 (Figure 3) and this was relatively sharp, activity being almost totally lost at or above pH 8.0 and at or below pH 6.5.

Subcellular fractionation of brain homogenates revealed that DFMO-sensitive ornithine decarboxylation activity was present in various cell components (Table II). The activity in the synaptosomal fraction was higher than in mitochondria while nuclear activity was lower and the myelin fraction possessed almost no ODC activity.

The 'mitochondrial' and 'synaptosomal' fractions are enriched in these components but are very heterogeneous; but, the nuclear fraction, under phase contrast microscopy, consists of relatively pure nuclei with few contaminants (10). The exact tonicity of the final incubation mixture was not completely constant. However, since each fraction was diluted at least 20-fold in the low ionic strength incubation medium prior to ODC assay, major lysis of organelles can be expected to have taken place.

Kinetic analysis of the DFMO-sensitive decarboxylation activity in the brain particulate fraction in the presence of 5×10^{-6} M AOAA revealed a K_m value of 15.7×10^{-6} M and a V_{max} of 29.6 μmol CO_2 produced/g tissue per hr. The corresponding values for soluble ODC were a K_m of

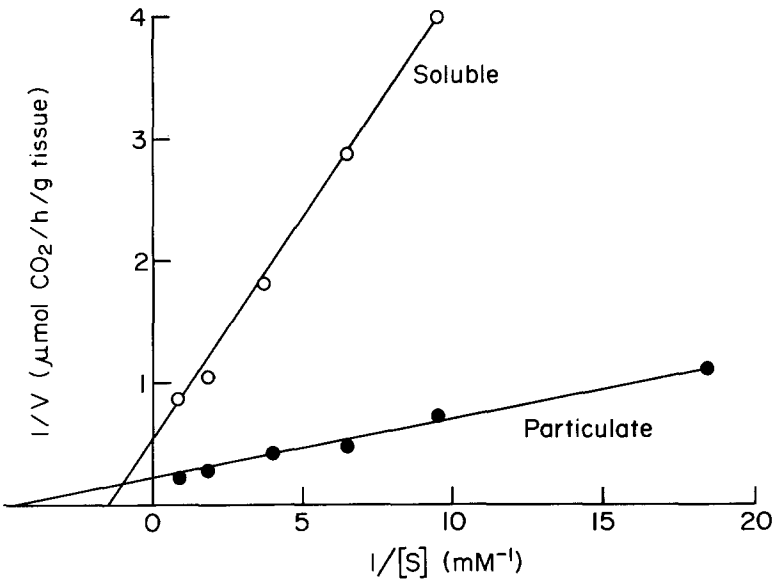

FIG. 4. Double reciprocal Lineweaver-Burke plot of l-ornithine concentration vs. ODC activity of cerebral soluble and particulate fractions. Each point represents the mean of three separate determinations and analysis was by least-squares regression of the linear bireciprocal relation. Soluble: K_m = 68 μM, V_{max} = 1.5 μmol CO_2/g tissue per hr. Particulate: K_m = 15 μM, V_{max} = 30 μmol CO_2/g tissue per hr. Coefficient of correlation was >0.98 for each line. Incubation was in the presence of 5×10^{-6} M AOAA.

67.7×10^{-6} M and a V_{max} of 1.5 μmol CO_2/g tissue per hr (Figure 4). These values for soluble brain ODC are similar to those reported previously by Butler and Schanberg (16).

DISCUSSION

The results suggest that the primary means of decarboxylation of ornithine in mature brain is by way of particulate rather than soluble enzymes. This decarboxylation cannot be primarily attributed to other catabolic pathways involving transamination and decarboxylation. The direct demonstration of putrescine formation suggests the existence of an insoluble form of ODC. This enzyme has a pH optimum similar to that of soluble ODC (17) and is most prevalent in the crude synaptosomal fraction. Although synaptosomes contain mitochondria, this finding implies a non-mitochondrial location for some synaptosomal ODC. The existence of synaptosomal ornithine decarboxylase has recently been re-

ported (18) using the production of [^{3}H]putrescine from ornithine as an index of activity.

The amino acid mixture and AOAA both inhibited particulate ODC. These compounds, at the concentrations used, also partially inhibit soluble ODC from kidney (16–22%, data not shown) and thus DFMO sensitivity may be a true index of ODC in particulate fractions. A less likely possibility is that DFMO is not as selective in its activity as has been reported previously (8). The lack of additive effects of particulate ODC inhibition by AOAA and the amino acid mixture may be because they inhibit different points on the pathway leading to the conversion of the carboxyl group of ornithine to CO_2. This pathway involves the transamination of ornithine and the subsequent decarboxylation of glutamatic or other amino acids.

In view of the lower K_m and higher V_{max} of particulate ODC relative to soluble ODC, it is likely that in the adult brain, much of the decarboxylation of ornithine in vivo proceeds by way of the membrane-bound enzyme. The functional meaning of particulate ODC within synaptosomal and mitochondrial fractions is unknown. However, some conditions such as electroshock can greatly increase the activity of soluble ODC (19), while the level of insoluble ODC is not affected by this treatment (20). Thus basal cell polyamine needs may be met by particulate ODC while the rapidly inducible soluble ODC may be important in adaptive responses of nerve tissue.

ACKNOWLEDGMENT

I would like to thank Ms. Sabreen Rahmaan for skilled technical assistance, and Dr. P. McCann of the Merrell Research Center, Cincinnati, Ohio, for the DFMO used in this research.

REFERENCES

1. JANNE, J., HOLTTA, E., KALLIO, A., and KAPYAHO, K. 1984. Role of polyamines and their antimetabolites in clinical medicine. Special Topics in Endocrin and Metabolism. 5:227–293.
2. COUSIN, M. A., LANDO, D., and MOGUILEWSKY, M. 1982. Ornithine decarboxylase induction by glucocorticoids in brain and liver of adrenalectomized rats. J. Neurochem. 38:1296–1304.
3. NAWATA, H., YAMAMOTO, R. S., and PORNIER, J. A. 1980. Ornithine decarboxylase induction and polyamine levels in kidney of estradiol-treated castrated male rats. Life Sciences 26:689–698.

4. RUSSELL, D. H., and SNYDER, S. H. 1969. Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase. Proc. Nat. Acad. Sci. 60:1420–1427.
5. SHAW, G. G. 1979. The polyamines in the nervous system. Biochem. Pharmacol. 28:1–5.
6. SCHMIDT, G. L., and CANTONI, G. L. 1972. Adenosylmethionine in developing rat brain. J. Neurochem. 20:1373–1385.
7. SEILER, N., and SARHAN, S. 1980. On the nonoccurrence of ornithine decarboxylase in nerve endings. Neurochem. Res. 5:97–100.
8. METCALF, B. W., BEY, P., DANZIN, C., JUNG, M. J., CASARA, P., and VEVERT, J. P. 1978. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C.4.1.1.17). J. Amer. Chem. Soc. 100:2551–2553.
9. GRILLO, M. A., and FOSSA, T. 1983. Ornithine decarboxylation in rat liver nuclei. Int. J. Biochem. 15:139–141.
10. BONDY, S. C., ROBERTS, S., and MORELOS, B. S. 1970. The histone acetylating enzyme of the brain. Biochem. J. 119:665–672.
11. GRAY, E. G., and WHITTAKER, V. P. 1962. The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation. Anat. (Lond.) 96:79–88.
12. DODD, P. R., HARDY, J. A., OAKLEY, A. E., EDWARDSON, J. A., PERRY, E. K., and DELAUNOY, J. P. 1981. A rapid method for preparing synaptosomes: comparison with alternative procedures. Brain Research 226:107–118.
13. DJURHUUS, R. 1981. Ornithine decarboxylase assay based upon the retention of putrescine by a strong cation-exchange paper. Anal. Biochem. 113:352–355.
14. MURPHY, B. J., and BROSNAN, M. E. 1976. Subcellular localization of ornithine decarboxylase in liver of control and growth-hormone treated rats. Biochem. J. 157:33–39.
15. JUNG, M. J., and SEILER, N. 1978. Enzyme-activated irreversible inhibitors of 1-ornithine-2-oxo-acid aminotransferase. J. Biol. Chem. 253:7431–7439.
16. BUTLER, S. R., and SCHANBERG, S. M. 1976. Apparent kinetic differences between brain and liver ornithine decarboxylase. Life Sciences 18:759–762.
17. KAYE, A. M. 1984. Ornithine decarboxylase. Purification and properties of ornithine decarboxylase. Cell Biochem. Funct. 2:2–6.
18. IQBAL, Z., and KOENIG, H. 1985. Polyamines appear to be second messengers in mediating Ca^{2+} fluxes and neurotransmitter release in potassium depolarized synaptosomes. Biochem. Biophys. Res. Commun. 133:563–573.
19. PAJUNEN, A. E. I., HIETALA, O. A. VIRRANSALO, E. L., and PIHA, R. S. 1978. Ornithine decarboxylase and adenosylmethionine decarboxylase in mouse brain-effect of electrical stimulation. J. Neurochem. 30:281–283.
20. BONDY, S. C., MITCHELL, C. L., RAHMAAN, S. and MASON, G. 1985. Regional variation in the response of cerebral ornithine decarboxylase to electroshock. Neurochem. Int. (in press).