Biochemical Pharmacology, Vol. 26, pp. 935–938. Pergamon Press, 1977. Printed in Great Britain.

MULTIPLE MONOAMINE OXIDASE ACTIVITIES IN HETEROGENEOUS POPULATIONS OF MOUSE LUNG MITOCHONDRIA*

BRIAN M. GALLAGHER
Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973, U.S.A.

(*Received* 27 *May* 1976; *accepted* 23 *July* 1976)

Abstract—Both monoamine oxidase (MAO) A and B activities were almost exclusively found associated with mitochondrial fractions in mouse lung, and these activities could be partially separated on linear sucrose gradients. The peak MAO B activity measured by the deamination of β-phenylethylamine (PEA) was consistently found in a population of mitochondria sedimenting in a denser region of the gradient than peak MAO A activity for 5-hydroxytryptamine (5-HT). Clorgyline strongly inhibited deamination of 5-HT across the mitochondrial fractions, while deamination of PEA remained high. Pargyline blocked PEA deamination, while considerable activity remained for 5-HT. These results provide evidence for the possible existence of heterogeneous subpopulations of lung mitochondria differing in sedimentation behavior and containing monoamine oxidase with different substrate specificity and inhibitor sensitivity.

The mammalian lung has the capacity to remove several amines [1–4] from the pulmonary vascular space and rapidly deaminate them via monoamine oxidase (MAO) [monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4]. MAO exists not as a homogeneous enzyme but rather in several forms with different substrate specificity [5], inhibitor sensitivity [6], thermal stability [7], antigenic properties [8], and electrophoretic mobility [9]. The type A form of mitochondrial MAO, which deaminates 5-hydroxytryptamine (5-HT) and norepinephrine, is selectively inhibited by harmaline and clorgyline [10]. Type B MAO preferentially deaminates β-phenylethylamine (PEA) and benzylamine and in most species is selectively inhibited by pargyline and deprenyl [10]. Evidence for the existence of these multiple forms of MAO in the lung derive chiefly from studies with whole organ homogenates [11], lung mitochondria isolated by differential centrifugation [12], and intact perfused lung [13–15].

Mitochondria from different tissues are not homogeneous organelles, but rather exist as heterogeneous populations with different distribution in sucrose density gradients [16–19] and enzymatic activities [5, 19, 20–25]. In view of these recent findings and the seemingly important function of the lung in the removal and deamination of various amine substrates, it was of interest to investigate the possibility that the multiple forms of MAO observed previously may be associated with different mitochondrial populations in the lung.

MATERIALS AND METHODS

Adult male Swiss albino mice (BNL strain) were injected intraperitoneally 1 hr prior to sacrifice with 10 mg/kg of pargyline, 10 mg/kg of clorgyline or 0.9% saline. The animals were sacrificed by cervical fracture, the thorax was rapidly opened, and the lungs were perfused via the heart with cold 0.25 M sucrose in 0.1 M potassium phosphate buffer, pH 7.4, to remove excess blood from the vascular space. The lungs were dissected free from trachea, bronchi and external blood vessels, minced finely and homogenized in 4 vol. of the above buffer with a Potter–Elvehjem homogenizer (10–12 strokes) at 0°. The homogenate was centrifuged twice at 600 *g* for 10 min each, the pellets were discarded, and the supernatants were layered on 4.5 ml, 0.25 to 1.73 M continuous sucrose gradients in 0.1 M potassium phosphate buffer, pH 7.4, prepared by a previous method [26]. The gradients used were either exponential or linear. In some experiments, mitochondria were first isolated by centrifugation at 12,100 *g* for 10 min at 4° and washed two times by resuspension in 0.25 M sucrose–0.1 M phosphate buffer before being layered onto sucrose gradients. The gradients were centrifuged at 32,500 rev/min in a SW65Ti rotor for 60 min or 17 hr at 4°. Fractions were collected by bottom puncture and stored at −20°. No detectable loss of MAO activity was noted up to several weeks.

MAO activity was measured radiometrically by a method similar to that described previously [27, 28]. 5-Hydroxytryptamine binoxolate[2-^{14}C] (48.5 mCi/m-mole) and β-phenylethylamine[1-^{14}C]hydrochloride (9.86 mCi/m-mole), both purchased from New England Nuclear (Boston, MA), were checked for impurities by thin-layer chromatography and used as specific substrates for type A and B MAO respectively [5]. The reaction mixtures typically contained 25–50 μl of gradient fraction (10–120 μg protein), 33.7 μM ^{14}C-substrate in a total volume of 300 μl of 0.1 M potassium phosphate buffer, pH 7.4. The reaction mixtures (without substrates) were warmed at 37° for 10 min, the substrate was added, and the reactions

* Research performed under the auspices of the U.S. Energy Research and Development Administration. Portions of this work were presented at the Sixtieth Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, Calif.

were allowed to proceed for 25 min at 37°. The reactions were stopped with 200 μl of 2 N HCl, 3 ml ethyl acetate was added, and the deaminated metabolites were extracted by vigorous shaking for several min. The phases were separated by centrifugation at 1000 *g* for 5 min, and a 1-ml aliquot of the organic phase was counted in 10 ml Aquasol in a liquid scintillation counter. Samples were corrected for quenching by internal standardization. Blank reaction tubes containing boiled enzyme or no enzyme were handled identically to the samples. All enzyme determinations were carried out in duplicate. Greater than 85 per cent of the activity applied to the gradient was recovered in the fractions collected.

Cytochrome oxidase was measured [29] and protein was estimated in duplicate using bovine serum albumin as a standard [30]. Sucrose concentrations were measured with an Abbé refractometer.

RESULTS

In preliminary experiments, the subcellular localization of monoamine oxidase in lung homogenates was measured on exponential sucrose gradients designed to separate the mitochondrial fraction from the microsomal and soluble fractions (Fig. 1). The peaks for both A and B forms of monoamine oxidase and cytochrome oxidase, specific markers for outer and inner mitochondrial membranes [31], respectively, were coincident and occupied the denser regions of the gradient, suggesting minimal organelle damage during preparation. Some activity for these enzymes near the top (microsomal/soluble region) of the gradient may represent mitochondrial fragments and/or microsomal MAO activity. This activity could be eliminated by prior isolation and washing of the mitochondria. However, the majority of both mitochondrial MAO and cytochrome oxidase activities cosedimented during centrifugation on exponential gradients.

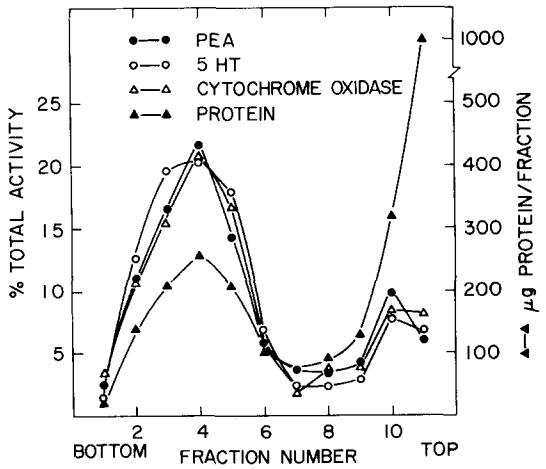

Fig. 1. Distribution of MAO A, B, cytochrome oxidase and protein from whole lung homogenates separated on exponential sucrose gradients. Enzymatic activities are plotted as per cent total activity recovered from gradients (>85 per cent in all cases) and are the average of duplicate determinations.

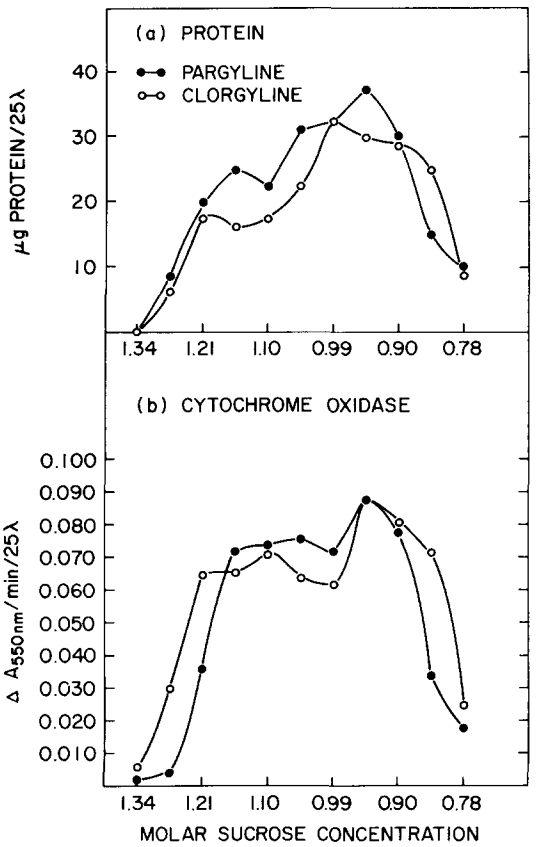

Fig. 2. (a) Mitochondrial protein distribution in linear sucrose gradients for mitochondria isolated from animals pretreated with pargyline or clorgyline (10 mg/kg). (b) Cytochrome oxidase distribution in mitochondrial fractions from animals pre-treated with pargyline or clorgyline.

When linear gradients were used, the mitochondrial protein peaks (Fig. 2a) and cytochrome oxidase activities (Fig. 2b) became somewhat heterogeneous but correlated well between animals pre-treated with pargyline or clorgyline. The MAO activities did not, however, follow this pattern (Fig. 3). The MAO activity for 5-HT in mitochondria isolated from animals treated with pargyline (Fig. 3a) remained high and consistently peaked in a less dense region of the gradient, whereas the activity in the same density fractions from animals treated with clorgyline was strongly inhibited. The MAO activity toward PEA was almost completely blocked by prior treatment of animals with pargyline, but remained high in mitochondria isolated from clorgyline-treated animals (Fig. 3b). Furthermore, a peak of PEA-deaminating activity was consistently found at significantly higher sucrose density. The total lung PEA-deaminating activity was higher than 5-HT-deaminating activity in all preparations from mouse lung.

When the data were plotted as specific activities to compensate for differences in the mitochondrial protein distribution across the gradient, both types of MAO activities were heterogeneous (Fig. 4). The absolute pattern varied with the number of fractions collected and with different preparations; however, the peak MAO B activity was consistently at a denser region of the gradient than peak MAO A activity.

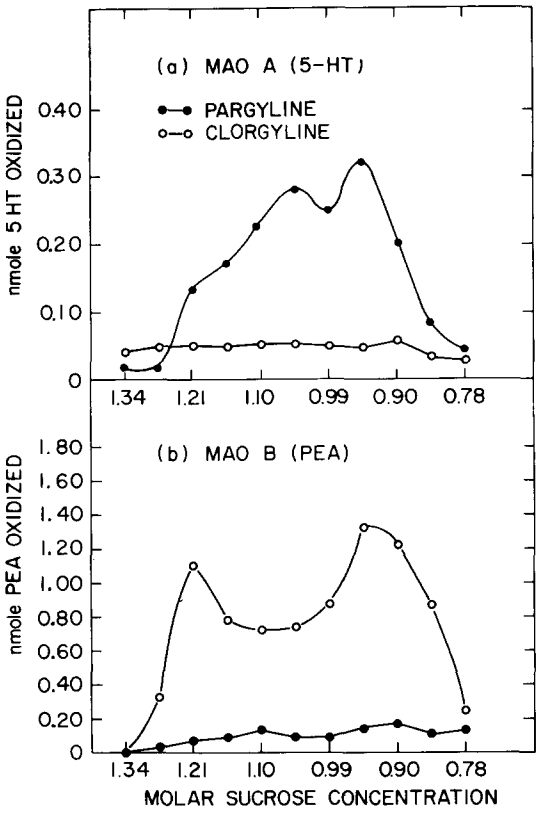

Fig. 3. (a) MAO A activity in mitochondria on linear gradients using [^{14}C]5-HT as substrate from animals treated with pargyline or clorgyline (10 mg/kg). (b) MAO B activity measured with [^{14}C]PEA in mitochondria from animals treated with pargyline or clorgyline.

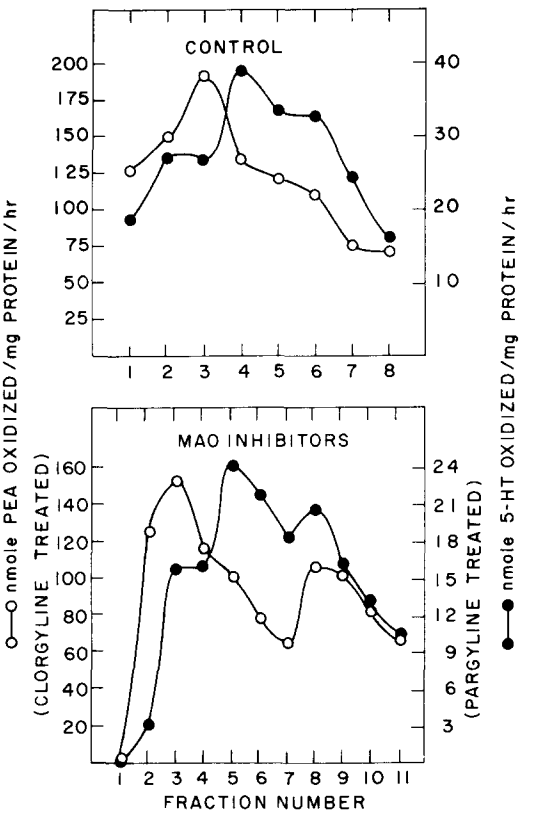

Fig. 4. Mitochondrial MAO distribution on linear sucrose gradients plotted according to specific activities in saline control or drug-treated animals.

The partial separation of activities was more pronounced in some preparations and became sharper in samples centrifuged 17 hr under conditions that allow for separation by buoyant density (Fig. 5). Thus, the 1-hr separation of activities may reflect a combination of differences in size, shape and density.

DISCUSSION

These results are the first report of the possible existence of heterogeneous subpopulations of lung mitochondria having a differing sedimentation pattern, MAO inhibitor sensitivity and MAO substrate specificity. Partial separation of MAO A and MAO B activities has been previously demonstrated in rat brain preparations [5]. As in the present results, the highest activity toward the MAO B substrate, PEA, was located in denser regions of the gradient than peak MAO A activity. However, brain tissue provides several problems during homogenization in isotonic sucrose in that the pre-synaptic mitochondria are largely enclosed in particles (synaptosomes) of varying size. This size variation depends on the amount of associated membrane material whereas the free mitochondria, believed to be of glial origins, lack this association [21]. Thus, the observed differences in the sedimentation pattern of brain mitochondria may reflect primarily differences in contaminating membranous material.

Much of the difficulty in demonstrating multiple MAO species has resulted from the use of "purified" MAO preparations [31, 32] and arises from differences in the techniques used for solubilization and purification. Although high sucrose concentrations used in density gradient techniques can alter mitochondrial structures [33], intact mitochondria may provide a more useful system for study. Such an approach has met with some success in separating brain mitochondria having different MAO activities [21, 33].

The presently observed differences in the MAO activities of lung mitochondria in sucrose gradients might result from membrane contaminants, but it seems unlikely that such contaminants could simultaneously and consistently confer substrate and inhibitor specificities and also determine the characteristic sedimentation patterns. This is particularly true in light of recent evidence that both the substrate and inhibitor selectivities reported here were also found in intact perfused rat and rabbit lungs [13–15]. The finding [15] that the deamination of 5-HT and PEA by lung was not significantly influenced by 200-fold molar excess of the competing substrate strongly supports the evidence for separate forms of lung mitochondrial MAO. The present findings are consistent with these reports and suggest that these enzymatic activities may be associated with distinct subpopulations of lung mitochondria.

The cellular origin of the mitochondria used in these studies is not known since the lung contains

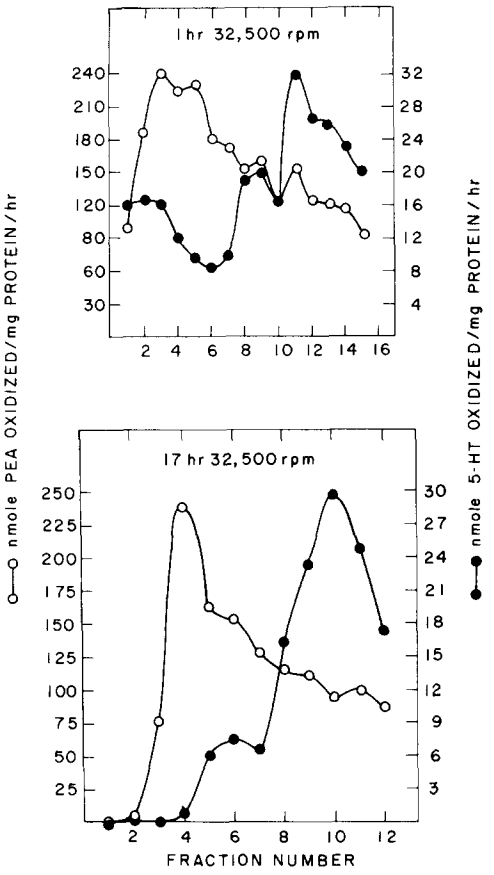

Fig. 5. MAO A and B activities of lung mitochondria centrifuged for 1 or 17 hr at 32,500 rev/min (SW65Ti rotor) on linear gradients.

some 42 cell types [34], but autoradiographic studies have implicated the capillary endothelial cells as the sites of amine degradation in lung [35–37]. These studies have used labeled MAO substrates and MAO inhibitors to slow the metabolism of these highly diffusible molecules. In view of the present findings on inhibitor selectivity and the previous reports demonstrating the use of specific irreversible MAO inhibitors as MAO titrants, studies are currently in progress with labeled inhibitors to determine the cellular origins of the MAO activities responsible for the deamination of 5-HT and PEA.

Acknowledgements—Pargyline·HCl and clorgyline·HCl were provided by Abbott Laboratories and May & Baker, Ltd. respectively. I express my appreciation to Drs. Richard Ehrenkaufer and David Lloyd for helpful discussions during this work and to Dr. A. P. Wolf for reading the manuscript.

REFERENCES

1. J. R. Vane, *Br. J. Pharmac.* **35,** 209 (1969).
2. C. N. Gillis, *Anesthesiology* **39,** 626 (1973).
3. V. A. Alabaster and Y. S. Bakhle, *Br. J. Pharmac.* **40,** 468 (1970).
4. J. S. Fowler, B. M. Gallagher, R. R. Mac Gregor and A. P. Wolf, *J. Pharmac. exp. Ther.* **198,** 133 (1976).
5. H. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187,** 365 (1973).
6. J. P. Johnston, *Biochem. Pharmac.* **17,** 1285 (1968).
7. R. F. Squires, *Biochem. Pharmac.* **17,** 1401 (1968).
8. B. K. Hartman and S. Udenfriend, *Adv. Biochem. Pharmac.* **5,** 119 (1972).
9. M. B. H. Youdim, G. G. S. Collins and M. Sandler, *Nature, Lond.* **223,** 626 (1969).
10. R. F. Squires, in *Adv. Biochem. Psychopharmac.* (Eds. E. Costa and M. Sandler), Vol. 5, p. 355. Raven Press, New York (1972).
11. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **23,** 1138 (1974).
12. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **23,** 2537 (1974).
13. C. N. Gillis, J. A. Roth and K. R. Baker, *Chest* **67,** 26S (1975).
14. J. A. Roth and C. N. Gillis, *J. Pharmac. exp. Ther.* **194,** 537 (1975).
15. Y. S. Bakhle and M. B. H. Youdim, *Br. J. Pharmac.* **56,** 125 (1976).
16. R. W. Swick, J. L. Strange, S. L. Nance and J. F. Thompson, *Biochemistry* **6,** 737 (1967).
17. L. Salganicoff and E. De Robertis, *J. Neurochem.* **12,** 287 (1965).
18. A. Neidle, C. J. Van der Berg and A. Grynbaum, *J. Neurochem.* **16,** 225 (1969).
19. G. G. D. Blokhuis and H. Veldstra, *Fedn Eur. Biochem. Soc.* **11,** 197 (1970).
20. A. Hamberger, C. Blomstrand and A. L. Lehninger, *J. Cell Biol.* **45,** 221 (1970).
21. M. C. Kroon and H. Veldstra, *Fedn Eur. Biochem. Soc. Lett.* **24,** 173 (1972).
22. M. A. Wilson and J. Cascarano, *Biochem. J.* **129,** 209 (1972).
23. G. Weiss, C. Lamartimere, B. Muller-Ohly and W. Seubert, *Eur. J. Biochem.* **43,** 391 (1974).
24. C. Lamartimere and G. Weiss, *Hoppe-Seyler's Z. physiol. Chem.* **355,** 1549 (1974).
25. C. Lamartimere and G. Weiss, *Hoppe-Seyler's Z. physiol. Chem.* **356,** 1079 (1975).
26. J. De Champlain, R. A. Mueller and J. Axelrod, *J. Pharmac. exp. Ther.* **166,** 339 (1969).
27. R. J. Wurtman and J. Axelrod, *Biochem. Pharmac.* **12,** 1439 (1964).
28. R. E. McCaman, M. W. McCaman, J. M. Hunt and M. S. Smith, *J. Neurochem.* **12,** 15 (1965).
29. S. J. Cooperstein and A. Lazarow, *J. biol. Chem.* **189,** 665 (1951).
30. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
31. C. Schnaitman and J. W. Greenawalt, *J. Cell Biol.* **38,** 158 (1968).
32. K. Tipton, M. Houslay and N. Garrett, *Nature New. Biol.* **246,** 213 (1973).
33. M. B. H. Youdim, *Biochem. Soc. Trans.* **1,** 1126 (1973).
34. E. A. B. Brown, *Drug Metab. Rev.* **3,** 33 (1974).
35. J. Hughes, C. N. Gillis and F. E. Bloom, *J. Pharmac. exp. Ther.* **169,** 237 (1969).
36. J. M. Strum and A. F. Junod, *J. Cell Biol.* **54,** 456 (1972).
37. S. A. M. Cross, V. A. Alabaster, Y. S. Bakhle and J. R. Vane, *Histochemistry* **39,** 83 (1974).