Biochemical Pharmacology, Vol. 27, pp. 1889–1893.

0006-2952/78/0801-1889$02.00/0

COMMENTARY

SUBSTRATE-SELECTIVE MONOAMINE OXIDASES— INHIBITOR, TISSUE, SPECIES AND FUNCTIONAL DIFFERENCES

DENNIS L. MURPHY
Clinical Neuropharmacology Branch, National Institute of Mental Health, Bethesda, MD 20014, U.S.A.

The oxidative deamination of neurotransmitter amines and other monoamines is now believed to be accomplished by two or more functionally different forms of monoamine oxidase (MAO). The comparative features of these enzyme forms have generated considerable recent attention for several reasons. First, different neuroamines are principally inactivated by one form and not the other. For example, norepinephrine and, in many tissues, serotonin, are primarily inactivated by MAO-A, while phenylethylamine is an MAO-B-selective substrate; dopamine, however, is a substrate for both the MAO-A and MAO-B forms. Second, inhibitors of high specificity for the MAO-A or MAO-B forms have been developed and have recently begun to be evaluated as adjuncts in the treatment of Parkinson's disease, depression and other disorders, in the hope that more selective clinical effects and/or lesser toxicity might be found. Third, increasing information on the differential localization of MAO-A and MAO-B in various tissues, brain areas, cell lines and sub-cellular preparations offers the possibility of better understanding the functional roles of MAO in detoxification, in the regulation of cellular amine levels and amine synthesis, and in "false transmitter" effects.

The existence of multiple monoamine oxidases became a prominent field for investigation in the last decade after Johnston's description of two forms of the enzyme differing in sensitivity to inhibition by clorgyline [1] and after a series of reports demonstrating the separation of three to five distinct bands of MAO activity using polyacrylamide gel electrophoresis [2]. The electrophoretically separable bands are now thought to represent artifacts resulting either from procedures required to solubilize this membrane-bound enzyme or from the electrophoretic technique itself [3]. Nonetheless, much additional data from inhibitor specificity, substrate selectivity, tissue localization and other properties have continued to suggest the existence of MAO-A and MAO-B forms. Two books [4, 5] and several articles [3, 6, 7] provide detailed reviews of some of this information.

The question of the existence of substrate-selective monoamine oxidases is not new. In the 1928 report first describing MAO, Hare [8] named the enzyme she discovered in rabbit liver, tyramine oxidase, and noted that it did not oxidize adrenaline. An adrenaline oxidase was next described [9], as was an aliphatic amine oxidase [10]. Blaschko *et al.* [11] investigated multiple substrates (including tyramine, adrenaline and phenylethylamine) in liver, brain and other tissues from mammals, other vertebrates, invertebrates and other animals and plants (but not the rabbit) and concluded in 1937 that these three oxidases "are similar in every property that has been investigated, and it is therefore suggested that they represent one enzyme which may be called 'amine oxidase'". This view remained predominant until recently. The history of MAO might have been different, however, if Hare's original tissue source of tyramine oxidase had been included in these studies. A 1978 report by Edwards and Malsbury [12] indicates that rabbit liver and brain possess a 5-fold higher ratio of MAO-B to MAO-A activity than rat liver and brain; observations of the enzyme activity ratio differences in the 1937 experiments for adrenaline vs phenylethylamine or adrenaline vs tyramine might well have fostered further inquiry into the possible plurality of monoamine oxidase 40 years ago.

The exact nature of the functionally different MAO forms remains under intensive investigation, and the two-form hypothesis remains an imperfect model. Many of the functional differences between MAO-A and MAO-B appear to depend upon maintenance of the integrity of the enzyme in the outer mitochondrial membrane. Solubilization and delipidation procedures variably alter properties of the enzyme, including substrate selectivity [3, 13]. Thus, indirect methods such as the use of relatively specific inhibitors, selective substrates or immunologic techniques have provided most of the evidence suggesting the existence of two or more different enzymes or, according to some interpretations, two or more different sites on the same enzyme. The mitochondrial monoamine oxidases (which are believed to be synthesized by the endoplasmic reticulum under nuclear DNA control, and later become attached to the outer mitochondrial membrane [14]) utilize FAD as a catalytic center cofactor. They have long been differentiated from histaminase and other diamine oxidases, and from the soluble amine oxidases found in cardiovascular tissues, dental pulp and plasma which utilize copper and possibly pyridoxal as cofactors [15]. These latter enzymes share some inhibitor sensitivities and possess some common substrates with the mitochondrial-FAD forms of MAO, but are distinguished by their sensitivity to carbonyl reagents and by other properties. Their functional significance remains uncertain, as

does their exact relationship to the mitochondrial forms of MAO; neither they nor the diamine oxidases will be discussed further here.

Inhibitor-based characterization of mitochondrial forms of MAO. Johnston [1] first reported that graphing the percentage inhibition of tyramine deamination which was produced by increasing concentrations of clorgyline yielded biphasic plots for rat liver and several other tissues, and postulated the existence of two MAO species (enzymes A and B) differing over 100-fold in their sensitivity to this drug. While most other substrates examined in his and subsequent studies yielded biphasic plots, several monoamines exhibited single, sigmoid-shaped plots. For example, serotonin and norepinephrine were completely inhibited at low clorgyline concentrations ($< 10^{-7}$ M) in rat tissues, while benzylamine and phenylethylamine were sensitive to high drug concentrations only ($> 10^{-5}$ M) [2–6]. Opposite selectivity is shown by two other propargylamine compounds, deprenyl and pargyline, which inhibit the substrates benzylamine and phenylethylamine (preferential substrates for MAO-B) at lower drug concentrations than are required for the inhibition of serotonin deamination.

Other preferential MAO-A inhibitors include harmine and harmaline, a series of cyclopropylamine-substituted oxadiazoles and another series of *N*-substituted cyclopropylamines, especially Lilly 51641 [2–6, 16, 17]. Two other drugs from this latter series, Lilly 54761 and Lilly 54748, show preferential MAO-B inhibition [17]. With the exception of harmine and harmaline, all of these agents including the propargylamines are irreversible inhibitors. An initial time-dependent stage studied for the propargylamines, however, involves an interaction with the catalytic site of the enzyme and is a competitive, reversible reaction which differs for the MAO-A and MAO-B forms and correlates with their specificity [3, 18]. Differential lipid solubility also contributes to the inhibitory potency of propargylamine compounds [19], a finding that may also be pertinent to the observations that MAO-A is markedly more sensitive to inactivation by methyl ethyl ketone delipidation than is MAO-B [13].

Substrate selectivity of the monoamine oxidases. Since almost all tissues contain mixtures of clorgyline-sensitive and -insensitive MAO activities, lists of preferred substrates for the MAO-A and MAO-B forms have generally been obtained from studies of specific inhibitor and substrate interactions [2–6]. Many substrates have not yet been adequately studied, and it is not clear precisely what characteristics of the different monoamines contribute to their preferential deamination by MAO-A or MAO-B. In general, substrates with more polar aromatic rings tend to be better MAO-A substrates [6], a conclusion in keeping with the suggested lipid environment differences for the MAO-A vs MAO-B sites.

The general outline of substrates preferred by MAO-A and MAO-B noted above has been derived primarily from studies of rat liver and brain. It is now clear that extrapolations cannot always be made to other species and tissues. For example, serotonin deamination is sensitive, in part, to intermediate concentrations of deprenyl in pig, monkey and human brain, and in cat, dog and rabbit liver (Ref. 3; D. L. Murphy, unpublished observations). While ratios of serotonin to phenylethylamine deamination have been used as indices of the relative proportions of MAO-A and MAO-B activities in some studies, it would seem that this procedure is potentially inaccurate in non-rodent tissues in the absence of combined substrate–inhibitor studies. Even in rat brain, some evidence suggests that MAO-A is primarily responsible for dopamine deamination in the corpus striatum, while in whole rat brain homogenates dopamine is deaminated by both MAO-A and MAO-B [20, 21].

Kinetic studies of the inhibition of selective substrates by other substrates have yielded complex results. Observations of various types of competition between amines have led to debate about whether a more appropriate model might be provided by two interactive catalytic sites on a single enzyme than the postulation of two or more separate enzyme species [3, 7]. According to the two-site suggestions, interactions affecting site conformation, substrate specificity and other features of catalytic activity may provide the best explanation for the complex results observed. For example, it has been suggested that some data would be most readily interpretable if it were assumed that the preferred substrates for the catalytic site of one enzyme form acted as reversible inhibitors of the other site, with K_i values similar to their K_m values as substrates [22]. In studies directed toward MAO-A vs MAO-B differences, most substrates have been found to act as inhibitors of the relatively selective substrates, although a lack of cross-inhibition by phenylethylamine and serotonin has been reported [23]. Other substrate competition studies have demonstrated a more complex pattern of interactions for phenylethylamine deamination than for serotonin deamination [24, 25].

Tissue, cell, species and other differences suggestive of multiple forms of MAO. The recent identification of several tissues which possess properties of either MAO-A alone (e.g. mouse neuroblastoma and other cell lines grown in tissue culture) [26–28] or MAO-B alone (e.g. human platelet) [29] has reemphasized the likelihood of independent MAO forms. In fact, earlier studies demonstrating a selective loss of MAO-A activity after denervation had suggested that sympathetic neurons might contain predominantly MAO-A, and the denervated pineal gland predominantly MAO-B [30, 31]. Similarly, studies of different mitochondrial populations and synaptosomes had suggested that MAO substrate- and inhibitor-related properties might reflect anatomical specialization [32, 33]. Data obtained for complex tissues such as the liver, which contains more than forty different cell types, may not provide a sound basis for postulating the existence of multiple catalytic sites on the same enzyme rather than separate enzyme forms.

Whether the functionally differentiable MAO forms represent independent gene products is not known. Evidence that catalytically active monoamine oxidases may be dimers or polymers [34], and additional evidence from electrophoretic

studies [35], suggest the possibility that the monoamine oxidases may be composed of subunits analogous to those described for the lactate dehydrogenases [36]. Since several observed features of substrate and inhibitor interactions are most readily understandable on the basis of lipid contributions to MAO function [13], it may be that outer mitochondrial membrane characteristics affect enzyme conformation and confer some of the observed cell- or tissue-related specificity upon one or more common MAO forms [37].

The different sequences of appearance of MAO-A and MAO-B during the first weeks of development in the rat [38] have been interpreted as suggesting separate genetic regulation for the two enzyme forms. However, maturation of membrane or other cellular elements interacting with a common enzyme could provide an alternative explanation. Several immunologic approaches to the multiple MAO form question have provided evidence for two distinct MAO enzymes in bovine brain, only one of which is found in bovine liver [39], and some evidence that this brain-specific enzyme's properties are quite similar to those of the MAO-A form [40].

Several hormones known to affect MAO activity have recently been shown to alter MAO-A preferentially. Estradiol reduces MAO activity in the rat hypothalamus and amygdala when the substrate is serotonin but not phenylethylamine; this effect is associated with an increased rate of degradation of MAO-A [41]. L-Thyroxine reduces rat liver MAO activity when either serotonin or benzylamine is used as the substrate. In the hearts of young rats, however, this hormone elevates MAO activity toward serotonin, while reducing MAO activity measured with benzylamine. L-Thyroxine treatment was also associated with changes in K_m in both heart and liver for serotonin but not for phenylethylamine deamination [42]. In other studies, serotonin deamination was preferentially sensitive to agents which reduced SH-group availability, while phenylethylamine deamination was relatively insensitive [43].

Limitations in the MAO-A and MAO-B model. It now seems likely that substrate selectivity for MAO-A and MAO-B is a relative rather than an absolute characteristic. Serotonin, for instance, is a very poor substrate for the human platelet MAO-B enzyme, but it is nonetheless deaminated to a slight extent by this tissue, as is phenylethylamine by the MAO-A in rodent neuroblastoma and glioma cells [26–29]. Similarly, while selective inhibitors may differ by over 500-fold in their capacity to inhibit one form compared to the other, such agents are very effective inhibitors of both forms when used in sufficient concentrations *in vitro* or *in vivo*. Examples of partial discrepancies in specificity are being observed as additional tissues, species and experimental conditions are explored. In the absence of more explicit molecular evidence on the nature of the MAO forms, combinations of preferred substrates and selective inhibitors are now being used to validate and quantitate MAO-A and MAO-B activities. However, technical and theoretical problems limit the confidence with which conclusions from specific activity ratio comparisons with different substrates can be held. Often, experimental conditions such as pH, buffer, assay techniques, protein concentrations or other factors are not equally applicable or optimal for the comparative study of different substrates or inhibitors [44–46]. Furthermore, issues such as aldehyde product binding, aldehyde dehydrogenase activity, and hydrogen peroxide effects are unstudied factors in many reports [47]. Nonetheless, while much remains unknown, the current aggregate of evidence strongly suggests that two MAO forms with quite distinctive properties exist separately in some tissues and together in others. As indicated above, it has not been resolved whether these forms at the molecular level represent distinct enzymes, polymers or different conformations of the same enzyme, or the same enzyme in different membrane environments. Data indicating differing characteristics of the catalytic centers and binding sites for the two enzyme forms are emerging rapidly, and perhaps may be further resolved with the use of approaches permitting the probing of features of these sites within the intact membrane such as electron spin resonance techniques and the use of inhibitors with fluorescent substituents [48, 49].

Functional consequences of multiple forms of MAO. It has been questioned whether the differences in response to selective inhibitors and substrates observed *in vitro* are observable *in vivo*, since the irreversible nature of most inhibitors in use could permit cumulative inhibition of the relatively insensitive enzyme form as well as the more sensitive form [5]. Furthermore, there is some direct evidence that some of the inhibition specificity *in vitro* is lost under conditions which are more similar to those found *in vivo* [5, 16]. Nonetheless, dosages of 0.5 to 2 mg/kg of either clorgyline, deprenyl or pargyline have been shown to yield quite specific inhibition of either serotonin or phenylethylamine deamination *in vivo* [21, 50–52]. In addition, some expected functional correlates of this selective inhibition have been observed, including differential changes in the formation of deaminated catecholamine and serotonin metabolites, potentiation of phenylethylamine-induced stereotypy, and reversal of reserpine effects, including potentiation of the anti-reserpine effects of dopa [20, 21, 50–52]. One clorgyline–deprenyl comparison led to the unexpected conclusion that a serotonin-related hyperactivity syndrome seen in rats after tryptophan was potentiated only slightly by high doses of clorgyline, but was elicited by low doses of clorgyline and deprenyl given together. This suggested that the behavior in question might be most closely related to the accumulation of a methylated metabolite of serotonin which is a preferred MAO-B substrate [51]. It seems likely that some of the other biological and behavioral effects of MAO inhibition may turn out to be consequences not only of elevation of amine levels in cells but also of more complex events [5].

Studies with selective substrate–inhibitor interactions have also revealed species differences in the importance of MAO-A and MAO-B in the deamination of certain neurotransmitter amines. For example, in human brain, dopamine appears to be deaminated primarily by MAO-B [53], whereas in

the rat, dopamine inactivation is predominantly by MAO-A, with a small contribution from MAO-B [20, 21]. Fairly large species differences exist in the relative amounts of MAO-A and MAO-B estimated from tyramine–clorgyline plots in the brain of rat (55 and 45 per cent, respectively) non-human primate (20 and 80 per cent, respectively) and man (15 and 85 per cent, respectively) (Refs. 3 and 54, and D. L. Murphy *et al.*, unpublished observations). These differences suggest that many of the assumptions dependent upon a communality in amine changes in response to MAO inhibitors across species require re-evaluation.

Many other questions of functional significance remain open. First, are MAO-A and MAO-B differentially located in cell types according to metabolic needs? The presence of MAO-A in peripheral sympathetic neurons [30, 31] and the absence of any MAO activity in acetylcholine-containing synaptosomes [55] seem fitting, but what is the role of MAO-B? The B form appears to be most important in preventing the accumulation of extraneous amines and amine metabolites in cytoplasm and storage vesicles and thus avoiding "false transmitter"-type impairment, but does it have any more specific functions? Why is it the predominant MAO form in the human platelet and the pineal gland—two cell types noted for their serotonin-related functions? Second, is MAO-B (or MAO-A) in brain, liver and other tissues, such as the platelet, the same enzyme in terms of both molecular configuration and susceptibility to regulatory influences? This question is of practical as well as theoretical importance, since over forty papers in the last 5 years have evaluated correlations between human platelet MAO activity and disease states, behavioral patterns, and the clinical effects of MAO inhibitors—most of which have inferred some meaningful association between this measure and brain or other tissue MAO activity. This association remains undemonstrated. Third, are the MAOs present in large excess, as has usually been suggested, so that only marked MAO inhibition can yield physiological consequences, or are moderate drug-induced or endogenous reductions in MAO activity genuinely associated with biological and behavioral differences [56, 57]? Conversely, if the MAOs are present in excess, how can the suggested amine-related effects of higher-than-usual MAO activities be understood [58]? Fourth, are the MAOs important regulatory enzymes in neurotransmitter amine-containing cells [5], and, if so, are their functions modulated, as some data suggest, by steroid hormones? Fifth, are reductions in MAO activity significant elements in such diverse disorders as migraine, the Lesch–Nyhan syndrome, alcoholism and various psychiatric disorders such as schizophrenia and bipolar affective disorders [59–61]? Finally, can therapeutic agents with greater specificity and fewer adverse effects be derived from the newer biochemical pharmacology of substrate-selective MAO-inhibiting drugs?

REFERENCES

1. J. P. Johnston, *Biochem. Pharmac.* **17,** 1285 (1968).
2. M. Sandler and M. B. H. Youdim, *Pharmac. Rev.* **24,** 331 (1972).
3. M. D. Houslay, K. F. Tipton and M. B. H. Youdim, *Life Sci.* **19,** 467 (1976).
4. E. Costa and P. Greengard (Eds), *Monoamine Oxidase—New Vistas*, Raven Press, NY (1972).
5. G. E. Wolstenholme and J. Knight (Eds), *Monoamine Oxidase and Its Inhibition*, Elsevier, Amsterdam (1976).
6. N. H. Neff and H-Y. T. Yang, *Life Sci.* **14,** 2061 (1974).
7. M. Jain, *Life Sci.* **20,** 1925 (1977).
8. M. L. C. Hare, *Biochem. J.* **22,** 968 (1928).
9. H. Blaschko, D. Richter and H. Schlossmann, *J. Physiol., Lond.* **90,** 1 (1937).
10. C. E. M. Pugh and J. H. Quastel, *Biochem. J.* **31,** 286 (1937).
11. H. Blaschko, D. Richter and H. Schlossmann, *Biochem. J.* **31,** 2187 (1937).
12. D. J. Edwards and C. W. Malsbury, *Biochem. Pharmac.* **27,** 959 (1978).
13. B. Ekstedt and L. Oreland, *Biochem. Pharmac.* **25,** 119 (1976).
14. N. H. Neff and C. Goridis, *Adv. Biochem. Psychopharmac.* **5,** 307 (1972).
15. K. T. Yasunobu, H. Ishizaki and N. Minamiura, *Molec. cell. Biochem.* **13,** 3 (1976).
16. R. F. Long, T. J. Mantle and K. Wilson, *Biochem. Pharmac.* **25,** 247 (1976).
17. D. L. Murphy, C. H. Donnelly, E. Richelson and R. W. Fuller, *Biochem. Pharmac.* in press (1978).
18. T. Egashira, B. Ekstedt and L. Oreland, *Biochem. Pharmac.* **25,** 2583 (1976).
19. C. H. Williams and J. Lawson, *Biochem. Pharmac.* **24,** 1889 (1975).
20. P. C. Waldmeier, A. Delini-Stula and L. Maitre, *Naunyn-Schmiedeberg's Arch. Pharmak.* **292,** 9 (1976).
21. H-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **189,** 733 (1974).
22. M. D. Houslay and K. F. Tipton, *Biochem. J.* **139,** 645 (1974).
23. H. L White and J. C. Wu, *J. Neurochem.* **25,** 21 (1975).
24. D. J. Edwards and S-S. Chang, *Biochem. biophys. Res. Commun.* **65,** 1018 (1975).
25. B. Ekstedt, *Biochem. Pharmac.* **25,** 1133 (1976).
26. C. H. Donnelly, E. Richelson and D. L. Murphy, *Biochem. Pharmac.* **25,** 1639 (1976).
27. D. L. Murphy, C. H. Donnelly and E. Richelson, *J. Neurochem.* **26,** 1231 (1976).
28. M. Hawkins and X. O. Breakefield, *J. Neurochem.* in press (1978).
29. C. H. Donnelly and D. L. Murphy, *Biochem. Pharmac.* **26,** 853 (1977).
30. B. Jarrot, *J. Neurochem.* **18,** 7 (1971).
31. H-Y. T. Yang, C. Goridis and N. H. Neff, *J. Neurochem.* **19,** 1241 (1972).
32. A. D. Student and D. J. Edwards, *Biochem. Pharmac.* **26,** 2337 (1977).
33. F. Owen, R. C. Bourne, J. C. K. Lai and R. Williams, *Biochem. Pharmac.* **26,** 289 (1977).
34. M. B. H. Youdim and G. G. S. Collins, *Eur. J. Biochem.* **18,** 73 (1971).
35. J. M. Diaz Borges and A. D'Iorio, *Can. J. Biochem.* **51,** 1089 (1973).
36. N. O. Kaplan, J. Everse and J. Admiraal, *Ann. N.Y. Acad. Sci.* **15,** 400 (1968).
37. K. F. Tipton, M. D. Houslay and N. J. Garrett, *Nature New Biol.* **246,** 213 (1973).
38. S. Bourgoin, F. Artaud, J. Adrien, F. Hery, J. Glowinski and M. Hamon, *J. Neurochem.* **28,** 417 (1977).

39. B. K. Hartman, *Biol. Psychiat.* **4,** 147 (1972).
40. R. McCauley and E. Racker, *Molec. cell. Biochem.* **1,** 73 (1973).
41. V. N. Luine and B. S. McEwen, *J. Neurochem.* **28,** 1221 (1977).
42. J. H. Tong and A. D'Iorio, *Endocrinology* **98,** 761 (1976).
43. H. L. White, A. T. Glassman and T. L. Sata, *Pharmacologist* **19,** 225 (1977).
44. J. Southgate and G. G. S. Collins, *Biochem. Pharmac.* **18,** 2285 (1969).
45. M. H. van Woert and G. C. Cortzias, *Biochem. Pharmac.* **15,** 275 (1966).
46. C. J. Fowler, B. A. Callingham and M. D. Houslay, *J. Pharm. Pharmac.* **29,** 411 (1977).
47. F. Ungar, B. Tabakoff and S. G. A. Alivisatos, *Biochem. Pharmac.* **22,** 1905 (1973).
48. R. H. Huang and S. Eiduson, *J. biol. Chem.* **252,** 284 (1977).
49. R. R. Rando, *Molec. Pharmac.* **13,** 726 (1977).
50. C. Braestrup, H. Andersen and A. Randrup, *Eur. J. Pharmac.* **34,** 181 (1975).
51. R. F. Squires and J. B. Lassen, *Psychopharmacology* **41,** 145 (1975).
52. J. A. Fuentes and N. H. Neff, *Neuropharmacology* **14,** 819 (1975).
53. V. Glover, M. Sandler, F. Owen and G. J. Riley, *Nature, Lond.* **265,** 80 (1977).
54. R. J. Squires, *Adv. Biochem. Psychopharmac.* **5,** 335 (1972).
55. G. R. L. Arniaz and E. D. P. De Robertis, *J. Neurochem.* **9,** 503 (1962).
56. A. J. Christmas, C. J. Coulson, D. R. Maxwell and D. Riddell, *Br. J. Pharmac.* **45,** 490 (1972).
57. M. Buchsbaum, R. D. Coursey and D. L. Murphy, *Science, N.Y.* **194,** 339 (1976).
58. M. Sandler, S. B. Carter, M. F. Cathbert and C. M. B. Pare, *Lancet* **1,** 1045 (1975).
59. X. O. Breakefield, C. M. Castiglione and S. B. Edelstein, *Science, N.Y.* **192,** 1018 (1976).
60. D. L. Murphy, in *Monoamine Oxidase and Its Inhibition*, pp. 341–351. Elsevier, Amsterdam (1976).
61. M. Sandler, M. B. H. Youdim and E. Hannington, *Nature, Lond.* **250,** 335 (1974).