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COMMENTARY

NEW DIRECTIONS IN MONOAMINE OXIDASE A AND B

SELECTIVE INHIBITORS AND SUBSTRATES

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Background

The first indication that monoamine oxidase (MAO) might have an important function in central amine neurotransmission began with the observation that iproniazid, an antituberculous agent, brought about a "lightening effect" in patients, and the demonstration by Zeller *et al.* [1] of its highly potent MAO inhibitory action. This original observation was confirmed by others and led to the study which determined that MAO inhibition results in increased brain levels of noradrenaline and serotonin [2]. Subsequent findings provided the basis for the introduction of MAO inhibitors into psychotherapy of affective disorders (depression), which together with discoveries of chlorpromazine and reserpine opened new perspectives for the treatment of central nervous system diseases. The initial enthusiasm for iproniazid and other MAO inhibitors, developed in the late 1950s and early 1960s, was dashed by the discovery of their hepatotoxicity and ability to potentiate the sympathomimetic action of indirectly acting amines (e.g. tyramine) found in food stuff, with resultant hypertensive crises in patients on MAO inhibitor therapy. This phenomenon, known as the "cheese effect" (because of the prevalence of tyramine in certain cheeses and foods), was the main reason for the discontinuation of the use of MAO inhibitors in the treatment of depression [2–4].

An important series of steps occurred between 1962 and 1970, when the enzyme was purified from a number of sources, and several investigators provided evidence for the presence of two MAO forms having different substrate specificities and inhibitor sensitivities [5, 6]. Johnston [7] differentiated multi-

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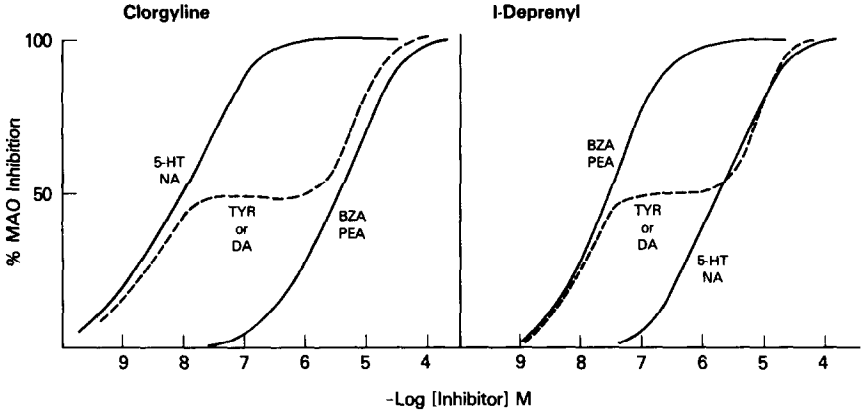

Fig. 1. Theoretical curves showing *in vitro* inhibitory activities of clorgyline and (−)-deprenyl using a variety of amine substrates, on an enzyme preparation containing MAO-A and MAO-B. The inhibition of tyramine (TYR) and dopamine (DA) oxidation by clorgyline and (−)-deprenyl reveals a pair of sigmoid curves joined by a horizontal section where the inhibition is invariant. The two sigmoid curves are attributed to the presence of two monoamine oxidases termed type A and type B. Type A is sensitive to clorgyline and oxidatively deaminates serotonin (5-HT), noradrenaline (NA), TYR and DA. Type B is resistant to clorgyline but sensitive to (−)-deprenyl and oxidizes phenylethylamine (PEA), benzylamine (BZA), TYR and DA, but not 5-HT or NA. The proportion of the two enzymes varies in a variety of tissues as can be demonstrated by the use of the A–B substrates TYR or DA employing clorgyline-induced inhibition. The level of the horizontal section indicates the ratio of the two enzymes [7].

Table 1. Distribution of MAO-A and -B in tissues of different species

Species and tissues	% of Total MAO activity A	B
Human		
Brain	20	80
Liver	55	45
Platelet	5	95
Placenta	>90	10
Intestine	75	25
Adrenal medulla	35	65
Chromaffin cell	<5	>95
Rat		
Brain	55	45
Liver	50	50
Intestine	70	30
Adrenal medulla	40	60
Cat		
Brain	25	75
Liver	35	65
Beef		
Liver	5	95
Pig		
Liver	20	80
Bovine		
Adrenal medulla	30	70

Table 2. Substrates of MAO-A and -B

Substrates	A	B	A–B
Adrenaline	+		
Noradrenaline	+		
Serotonin	+		
Octopamine	+		
Milacemide*		+	
Benzylamine		+	
Phenylethylamine		+	
Methyl-histamine		+	
N-Acetylputrescine		+	
MPTP*		+	
n-Pentylamine		+	
Decylamine		+	
Octylamine		+	
Tyramine			+
Dopamine			+
Tryptamine			+
Kynuramine			+
3-Methoxy-tyramine			+

* Milacemide, *n*-pentylaminoacetamide; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Table 3. Inhibitors of MAO-A and -B

Inhibitor	A	B	A–B	Clinical indications
Irreversible				
Clorgyline	+			Antidepressant
LY51641	+			
MDL-72145		+		
(−)-Deprenyl		+		Anti-parkinson and antidepressant
AGN 1135		+		
MDL-72145		+		
Pargyline		+		
Iproniazid			+	Antidepressant
Tranylcypromine			+	Antidepressant
Phenelzine			+	Antidepressant
Reversible				
Amiflamine	+			
Amphetamine	+			
Harmaline	+			
Moclobemide	+			Antidepressant
CGP-11305A	+			Antidepressant
Cimoxatone	+			
Toloxatone	+			Antidepressant
Ro 19-6327			+	Anti-parkinson?
Ro 16-6491			+	Anti-parkinson?
Milacemide			+	Anticonvulsant and antidepressant?

ple forms of MAO into subtypes A and B and described the first selective inhibitor of type A enzyme, clorgyline (Fig. 1). This classification has remained the basis for all the biochemical, pharmacological and physiological studies done on MAO since 1968 and was responsible for the identification of (−)-deprenyl in 1972 [8] as the selective inhibitor of type B enzyme, devoid of the "cheese effect." Since then, the distribution of the two MAO subtypes in different tissues and species has been established (Table 1) and the substrate (Table 2) and inhibitor (Table 3) specificities of these enzymes identified.

Pharmacological enzyme inhibitor–structure activity and radioligand studies using [^{14}C]clorgyline, [^{14}C]pargyline and [^{14}C](−)-deprenyl indicated that these inhibitors were bound to the same active sites on the two enzymes [9, 10]. Thus, the substrate and inhibitor specificities were associated with different recognition sites on their primary structures [11]. The inhibitor binding sites were shown to contain the pentapeptide Ser-Gly-Gly-Cys-Tyr in which the obligatory cofactor FAD is covalently bound through the thioether of cysteine to the 8α-position of the isoalloxazin moiety [12, 13]. These inhibitors bind stoichiometrically to N5 of FAD. Bach *et al.* [14], using oligonucleotide probes derived from sequenced peptide fragments, have isolated cDNA clones that encode the A and B forms of MAO. Both enzyme sequences contain the FAD pentapeptide but differ in primary amino acid sequences, indicating that MAO-A and -B are derived from separate genes [15] and are, therefore, under different control mechanisms, e.g. hormones [16].

The unique pharmacological activity of (−)-deprenyl as an inhibitor of MAO-B without the "cheese effect," together with the prevalence of MAO-B (for which dopamine and tyramine are substrates) in human basal ganglia [17], prompted Birkmayer and colleagues [18, 19] to investigate the drug clinically as an adjuvant to L-dopa for the management of Parkinson's disease. The potentiation of the anti-parkinson activity of L-dopa and the absence of the

cheese effect in (−)-deprenyl-treated patients were intriguing phenomena, and important steps for the future of MAO inhibitors as psychotropic drugs, since the irreversible selective MAO-A inhibitors, such as clorgyline, initiated the "cheese effect" [2–4]. The efforts in 1978–1982 were directed towards the search for other selective inhibitors devoid of the cheese effect. The development of other selective and potent reversible and irreversible inhibitors of MAO subtypes (Table 3), some of which have biochemical and pharmacological properties similar to (−)-deprenyl and clorgyline, led to the demonstration that the "cheese effect" was a property associated with inhibition of adrenergic intraneuronal MAO-A and not MAO-B [20]. These findings had a direct bearing on the future development of selective reversible MAO-A inhibitors. It was proposed that the indirectly acting sympathomimetic amine tyramine (MAO-A-B substrate) would displace the reversible inhibitor from the highly active intestinal MAO-A substrate binding sites and be deaminated by the enzyme [21]. Indeed, a reduced tyramine sensitivity and shorter duration of cheese effect was noted with this class of inhibitors after oral tyramine, in comparison with the irreversible inhibitors [22, 23]. By 1982, the success of (−)-deprenyl as an anti-parkinson agent was established. Its mechanism of action, however, has remained in doubt, even though it induces selective inhibition of MAO-B, and increases dopamine and phenylethylamine levels in the basal ganglia of parkinsonian brains obtained at autopsy [24, 25].

The future prospects for MAO-B inhibitors as anti-parkinson drugs were enhanced further in 1982 by the long-term retrospective clinical studies of Birkmayer *et al.* [26], showing longevity in (−)-deprenyl-treated parkinsonian subjects as confirmed by a larger study [27]. Similar observations have been published recently by Tetrud and Langston [28], who found that early initiation of (−)-deprenyl therapy in parkinsonian patients at first appearance of clinical signs of the disease prolonged the time required for initiation of L-dopa therapy. This phenomenon had not been described for any other anti-parkinson drug, and was attributed to the ability of (−)-deprenyl to inhibit brain MAO-B and prevent or retard the degenerative process of nigro-striatal dopamine neurons. The oxidation of amine substrates by MAO results in production of H_2O_2 and O_2 by the action of glutathione peroxidase. In basal ganglia of parkinsonian subjects, reduced content of the antioxidants ascorbic acid and reduced glutathione, and interaction with metal ions such as Fe^{2+} or Cu^{2+}, may lead to production of the cytotoxic hydroxyl radical ($^{\cdot}OH$) (see Ref. 29 for a more complete description). An alternative hypothesis was presented following the identification of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by Kopin and Markey and coworkers [30, 31], and its ability to induce parkinsonism and dopaminergic neurotoxicity in humans, monkeys and mice by Langston and coworkers [32]. The facts that MPTP is a substrate of MAO-B [33] and that selective MAO-B inhibitors prevent its metabolism to *N*-methyl-4-phenyl-1,2-dihydropyridiniumion (MPP^+) [33, 34] and its dopaminergic neurotoxicity have accorded a special role for MAO in the pathogenesis of Parkinson's disease. These findings have changed the views with regards to the origin and the long-term treatment of this disorder.

Current status and relevance of selective localization of MAO subtypes to pharmacology

(*a*) *Noradrenergic neurons.* Biochemical demonstration of the selective localization of MAO-A to noradrenergic nerves was made by Jarrott and Iversen [35] and Neff and coworkers [36]. These studies showed that MAO-A exists in both neuronal and extraneuronal peripheral tissues, but in nerve MAO-A forms the major component of MAO activity. Pharmacological confirmation of these findings was added by a number of studies showing that selective inhibition of MAO-A but not MAO-B led to potentiation of the pharmacological action of tyramine [37–41]. In the intact animal, potentiation of the effect of tyramine can result from either a decrease in extraneuronal metabolism of the amine or a selective inhibition of neuronal MAO. Decreased extraneuronal metabolism in gut and liver leads to increased blood levels of tyramine, whereas inhibition of neuronal MAO leads to an increase in the cytoplasmic pool of noradrenaline available for release by indirectly-acting sympathomimetic amines [39, 42]. As a result of the latter mechanism, the effect of indirectly-acting amines which are not substrates for MAO will also be potentiated, as will the effect of those indirectly-acting amines such as 2-phenylethylamine, which are selective substrates for MAO-B. Indeed, the cardiovascular effects of 2-phenylethylamine in the cat were potentiated to a greater extent by clorgyline than by (−)-deprenyl treatment [40]. Inhibition of neuronal MAO will also reduce intraneuronal tyramine metabolism, and increase the access of tyramine to vesicular noradrenaline stores [43]. Reversible inhibitors of MAO-A produced potentiation of the effects of tyramine administered intravenously in the rat, although to a lesser extent than that produced by clorgyline [41]. One reversible inhibitor of MAO-A, moclobemide, has been shown to produce only a minor degree of potentiation of tyramine pressor response in humans [44].

(*b*) *Effects in CNS.* Selective inhibitors of MAO-A have been shown to be effective antidepressant drugs [45–47]. An antidepressant effect of (−)-deprenyl, in dosage selective for MAO-B inhibitors, was not detectable in a group of patients suffering from endogenous depression [48]. When combined with 5-hydroxytryptophan, or with L-phenylalanine, however, (−)-deprenyl had a good antidepressant action, in a dose known to be selective for inhibition of MAO-B [49, 50]. When used alone, (−)-deprenyl may have a selective antidepressant effect in non-endogenous depressives [51].

By corollary with these clinical findings, inhibition of MAO-A but not MAO-B in rats is effective in reversing the actions of reserpine [52], and in causing the appearance of a behavioral hyperactivity syndrome similar to that seen following serotonin (5-HT) receptor agonists in rats given the selective inhibitor of 5-HT uptake, LM5008 [53].

An important consideration in the CNS action of

MAO inhibitors is the localization of subtypes of the enzyme to neuronal or glial cells, or in specific brain nuclei. A number of studies point to the localization of MAO-B in glial cells. In keeping with this initial finding, the proportion of MAO-B activity in rat brain was shown to increase following hemisection, or with aging [54].

The major advance in localization of brain MAO subtypes over the last few years has been the introduction of specific antibodies directed against the separate enzyme forms. The first demonstration of this type by Levitt *et al.* [55] showed a selective localization of MAO-B in astrocytes and serotonergic neurons in the rat brain, while catecholamine-containing cells of the substantia nigra and locus coeruleus did not stain for MAO-B. Cultured glial cells were also MAO-B positive. These observations were substantiated following development of monoclonal antibodies to both MAO-A and MAO-B [56, 57]. In rat, monkey and human brain tissue, MAO-B was found to be located in cell bodies of the raphe nucleus, as well as certain other cell groups of the hypothalamus and brain stem areas, and in astrocytes. Staining positive for MAO-A was found mainly in cell bodies of locus coeruleus and in other catecholaminergic cells of the brain stem, as well as light staining of substantia nigra and some astrocytes, particularly in the hypothalamus [55, 58, 59].

The observation that MAO-B is associated with nerve cell bodies of the raphe nucleus is of particular interest from two aspects: (a) the physiological importance of the MAO form with lower affinity for 5-HT being contained within serotonergic nerves, and (b) the pharmacological implications for the clinical application of selective MAO-B inhibitors in depression and other mental disorders. These and other aspects of MAO localization have been reviewed recently by several investigators [5, 60]. The occurrence of MAO-B within serotonergic nerves can be compared with the association of the same enzyme form with chromaffin cells [61] and viewed as a logical mechanism for conservation of amine by the neurons. Why, then, is MAO-A found within catecholaminergic nerves? The answer may indicate a more important role for a cytoplasmic pool of 5-HT in neuronal transmission than in the case of catecholaminergic nerves. Indeed, Kuhn *et al.* [62] have demonstrated that functional 5-HT release can be elicited from serotonergic nerves after reserpine treatment, provided that MAO is inhibited non-selectively. On the other hand, MAO-A may be associated with terminals of serotonergic neurons, and MAO-B in the cell bodies.

In attempting to study the metabolism of released monoamines, a model system has been developed using selective inhibitors of active uptake of 5-HT, noradrenaline or dopamine in a synaptosomal preparation. Metabolism of labeled amines with or without added uptake blocker indicates the difference between deamination by intra- and extraneuronal MAO [63]. Such determinations support the existence of a substantial pool of MAO-A within serotonergic synaptosomes from the hypothalamus, lending strength to the suggestion that subtypes of MAO may be expressed differently in cell bodies and nerve endings of serotonergic nerves arising from the raphe nuclei. These studies also indicated an important role of MAO-A located extraneuronally in the deamination of noradrenaline and dopamine in synaptosomes prepared from hypothalamus and striatum, respectively, lending substance to biochemical studies which show an important role of MAO-A in the deamination of striatal dopamine [63]. However, recent studies of Liccione and Azzaro [64] on the cyclic AMP-induced flux of dopamine indicate a significant extraneuronal metabolism by MAO-B, probably located in the glia. Indeed, Melamed [65] has stressed the significance of extraneuronal metabolism to the anti-parkinson action of L-dopa. The compartmentation of MAO-A and -B in the dopaminergic neurons and extraneuronal sites may very well explain why in the parkinsonian human brains the anti-parkinson drug (−)-deprenyl (MAO-B inhibitor) does not elevate brain dopamine (A–B substrate) levels as much as it does that of the selective MAO-B substrate 2-phenylethylamine [25].

Future directions

The original pharmacological identification of the two MAO subtypes, whose primary structures have now been identified by cDNA cloning and found to be different, has greatly advanced the knowledge of physiological aspects of these enzymes in relation to biogenic amine metabolism and function. MAO inhibitors (selective and non-selective) are now an important class of anti-depressant and anti-parkinson drugs. The major reason for their revival is the elimination of the severe side-effects in the newly developed drugs.

Future studies are needed to determine to what extent the cellular localization of MAO subtypes is related to physiological function of these cells. Thus, is the exclusive presence of MAO-B in platelets and adrenal chromaffin cells related to the ability of these cells to accumulate the highest concentrations of MAO-A substrates (serotonin and noradrenaline) and for what eventual physiological needs? For what purpose do the serotonergic nerve bodies of raphe exhibit the highest concentration of MAO-B in the brain as identified histochemically and how is the MAO subtype transferred to the A form as expressed in the axon terminal? Does this indicate a selective (axonal) transport process as proposed by Denney and Denney [60]? Are there other neurotoxins which are substrates of MAO-A or -B in a manner similar to that of MPTP, and will MAO inhibitors prevent or affect degeneration of nigro-striatal dopamine neurons of parkinsonian patients? The physiological regulation of the two enzyme forms and their response to MAO inhibitors are areas in which hard data are difficult to come by. However, recent culture studies, using cell types containing MAO-A or -B, have indicated that the regulation of these enzymes is under the control of different hormones [16]. Thus, although the synthesis of MAO-A can be up- or down-regulated by steroids, no such regulatory mechanism for MAO-B has been observed thus far. There is a clear need for investigation of this problem because of possible involvement of MAO-B in the pathogenesis of Parkinson's disease.

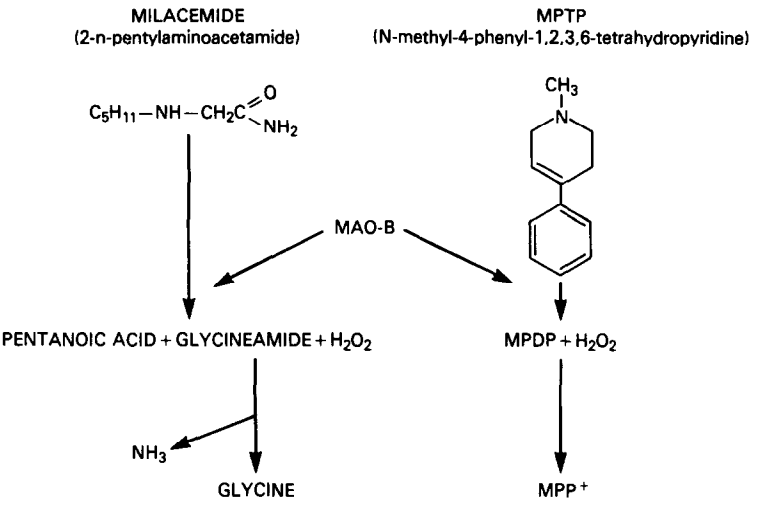

Fig. 2. Role of brain MAO-B in the oxidation of secondary (milacemide) and tertiary (MPTP) amines to neuroactive substances. MPDP = *N*-methyl-4-phenyl-dihydropyridine.

The future exciting prospects for neuropharmacology and neuropsychotherapy include the use of the oxidative reaction of MAO-B to deliver active compounds from prodrug substrates of MAO. This novel direction has come from recent studies showing that the pharmacologically active anticonvulsant, milacemide (*n*-pentylaminoacetamide), increases the brain inhibitory neurotransmitter, glycine. Milacemide, unlike glycine, readily crosses the blood–brain barrier and is metabolized to glycineamide and glycine by brain MAO-B ([66, 67]; Fig. 2). Its *in vivo* conversion, and ability to increase seizure threshold in animals, are inhibited by (−)-deprenyl but not by clorgyline [68]. Indeed, milacemide (a secondary amine) is a selective substrate [67] and an enzyme-activated specific inhibitor of MAO-B *in vitro* and *in vivo* [69]. This drug, similar to (−)-deprenyl, can inhibit specifically MAO-B in monkey brains and increase dopamine content but not serotonin or noradrenaline in the striatum [70]. The ability of MAO-B to convert inert amines such as MPTP and milacemide into neuroactive substances may not be totally unexpected (Fig. 2). Furthermore, putrescine, a diamine oxidase substrate, when N-acetylated in the brain, becomes a substrate of MAO-B (Youdim MBH, unpublished data). Oxidative deamination of *N*-acetylputrescine by MAO-B can lead to the formation of a second pool of γ-aminobutyric acid (GABA) [71]. The radioautographic binding studies of Da Prada and colleagues [72–74], using the highly selective reversible MAO-B inhibitor [^{3}H]Ro-19-6327, have indicated that this enzyme is far more richly and widely distributed in the rat and human brains as compared with the immunohistochemical methods using monoclonal antibodies. In addition, the enzyme is present in the substantia nigra, in various regions of the cerebellum, choroid plexus, and upper spinal cord and in other circumventricular regions. Thus, this enzyme may have some other unknown physiological functions and endogenous substrates. The reaction of MAO-B could thus be used to develop other prodrugs derived from amino acids or other agents for delivery into the brain.

Summary

Identification, cellular localization, and cDNA cloning of MAO subtypes A and B have increased the insight into the pharmacology of these enzymes, whose primary functions are intra- and extraneuronal inactivation of neurotransmitter (dopamine, noradrenaline and serotonin) and other biogenic amines. In addition, MAO oxidizes the inert uncharacteristic tertiary amine, MPTP, to the parkinson inducing dopaminergic neurotoxin, MPP^+, and the novel secondary amine anticonvulsant milacemide to the inhibitory amino acid neurotransmitter, glycine. These recent developments have provided new therapeutic perspectives for the management of Parkinson's disease and seizure disorders via the use of selective inhibitors and amino acid amine prodrug substrates of MAO-B.

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