Novel substrates and products of amine oxidase-catalysed reactions

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Introduction

Mitochondrial monoamine oxidase (MAO; EC 1.4.3.4) catalyses the oxidative deamination of a variety of primary, secondary and tertiary amines. Until very recently most of the attention concerning MAO activity was focused on the reaction of this enzyme to deaminate neurotransmitter substances, including adrenaline, noradrenaline, serotonin and dopamine. The ability of MAO to regulate the intraneuronal concentration of aminergic neurotransmitters and tissue concentrations of other sympathomimetic amines, such as tyramine, phenylethylamine and tryptamine, conferred a detoxifying role on this enzyme (see [1, 2] for reviews). However, more recent studies and developments in the reaction kinetics of MAO indicate that this enzyme may have other functional roles and, indeed, may be involved in converting rather inert tertiary amines, such as the Parkinson-inducing dopaminergic neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to the neuroactive metabolite MPP⁺ (N-methyl-4-phenyl-1,2-dihydropyridinium ion) [3]. Thus, MAO has also been assigned a role in the pathogenesis of Parkinson’s disease, resulting from its ability to convert (oxidize) environmental MAO-like neurotoxins to reactive metabolites. Although MPTP is a synthetic amine, such suggestions cannot be ignored, even though no such Parkinson disease-inducing environmental neurotoxin has so far been identified in the environment or the human brain.

In this paper we wish to describe a recently-discovered novel property of MAO involving its ability to convert novel inert secondary and tertiary amines into neuroactive substances with neurotransmitter properties, as a prerequisite for the treatment of neurological disorders of epilepsy, myoclonus, spinal spasticity, depressive illness and Parkinson’s disease.

Abbreviations used: MAO, monoamine oxidase; MPTP, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GABA, γ-aminobutyric acid; BBB, blood–brain barrier; NMDA, N-methyl-D-aspartate.

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Aliphatic and aromatic amine derivatives of glycine and γ-aminobutyric acid

γ-Aminobutyric acid (GABA) and glycine constitute the major inhibitory neurotransmitters in the brain [4]. Both neurotransmitters have been implicated in epilepsy, myoclonus, tardive dyskinesia, Huntington’s chorea, schizophrenia and even in Parkinson’s disease. However, neither amino acid crosses the blood–brain barrier (BBB). Although a limited number of metabolic inhibitors and agonists of GABA exist, so far no similar drugs exist for glycine. Thus the role of glycine in brain function and neurological diseases has been difficult to assess [4]. In normal circumstances, glycine is considered a major inhibitory neurotransmitter in the brain stem and upper spinal cord motor interneurons. Even so, it is now recognized that glycine also has an excitotoxic property in the hippocampus via its allosteric interaction with the N-methyl-D-aspartate (NMDA) receptor, a subtype of the glutamate receptor [5].

Milacemide (2-n-pentylaminoacetamide) is a secondary monoamine glycine derivative with a reported anticonvulsant activity equivalent to valproate in numerous animal models [6-9]. Unlike glycine it readily crosses the BBB, where its major pathway (> 90%) of metabolism is dealkylation to the metabolites glycineamide and glycine [6-10] (Fig. 1). The latter finding supports its clinical anticonvulsant activity in the absence of a sedative effect [8]. The compound is also thought to induce mood improvement and enhance cognition [8]. The formation and accumulation of glycine in forebrain, cerebellum, medulla and upper spinal cord but not in other tissues such as the liver, kidney and lung suggests a unique metabolic pathway related to its pharmacological profile.

The secondary amine nature of milacemide, together with involvement of oxidative reaction in its metabolism, suggests that the reaction may involve MAO activity in a similar manner to the original unsuspected ability of MAO to metabolize the dopaminergic Parkinson’s disease-inducing neurotoxin MPTP [3]. Indeed, we demonstrated, using numerous tissue preparations and animals, that milacemide is a selective MAO-B substrate
both in vitro and in vivo [10]. Thus selective MAO-B rather than MAO-A inhibitors can prevent its metabolism and anticonvulsant activity, suggesting that the formation of the inhibitory neurotransmitter, glycine, may be a prerequisite for its pharmacological activity as an anticonvulsant [11]. Nevertheless electrophysiological studies have demonstrated that milacemide itself is inhibitory to the excitatory and proconvulsant actions of the NMDA receptor [12]. However, neither milacemide nor its first metabolite glycinamide interact directly with the NMDA receptor in radio ligand binding studies (N. Harshak & M. B. H. Youdin, unpublished work).

Besides being a selective substrate of MAO-B with a relatively high affinity for this enzyme ($K_m = 30-80 \mu M$) as compared with MAO-A ($K_m = 1200-1500 \mu M$), milacemide also acts as a time-dependent, enzyme-activated, partially-reversible specific inhibitor of MAO-B [13] (Fig. 1). A reversible inhibition of MAO-A is also observed at high concentrations. Formation of a putative intermediate imine as an inhibitor bound reversibly to the enzyme has been suspected. Thus milacemide

### Table I

The kinetic parameters of oxidation of MAO-A and MAO-B substrates

Values for MAO substrates are compared with the newly-developed aliphatic and aromatic monoamine derivatives of glycine and γ-aminobutyric acid using guinea-pig brain mitochondrial preparation. n.d., not determined.

<table>
<thead>
<tr>
<th>Substrate (S)</th>
<th>MAO-A</th>
<th></th>
<th></th>
<th></th>
<th>MAO-B</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (m)</td>
<td>$K_m/(S)$</td>
<td>$V_{max}(S)/V_{max}(S-HT)$</td>
<td>$V_{max}(S)/V_{max}(S-HT)$</td>
<td>$K_m$ (m)</td>
<td>$K_m/(S)$</td>
<td>$V_{max}(S)/V_{max}(PEA)$</td>
<td>$V_{max}(S)/V_{max}(PEA)$</td>
</tr>
<tr>
<td>Serotonin</td>
<td>$4.4 \times 10^{-4}$</td>
<td>1</td>
<td>1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>β-Phenylethylamine</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>$8.8 \times 10^{-6}$</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milacemide</td>
<td>$1.4 \times 10^{-4}$</td>
<td>3.2</td>
<td>1.25</td>
<td>$2.0 \times 10^{-5}$</td>
<td>2.2</td>
<td>0.96</td>
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<td></td>
<td>$2.0 \times 10^{-5}$</td>
<td>0.08</td>
<td>2.25</td>
<td>$3.7 \times 10^{-5}$</td>
<td>4.3</td>
<td>0.08</td>
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<td>$3.3 \times 10^{-3}$</td>
<td>7.5</td>
<td>1.67</td>
<td>$1.2 \times 10^{-5}$</td>
<td>1.4</td>
<td>0.87</td>
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<td>4.0 $\times 10^{-3}$</td>
<td>90.9</td>
<td>1.00</td>
<td>$2.7 \times 10^{-2}$</td>
<td>310</td>
<td>0.49</td>
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<td></td>
<td>5.2 $\times 10^{-4}$</td>
<td>11.8</td>
<td>1.75</td>
<td>$&gt;2.4 \times 10^{-3}$</td>
<td>270</td>
<td>0.37</td>
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<tr>
<td></td>
<td>$&gt;1.0 \times 10^{-2}$</td>
<td>$&gt;227$</td>
<td></td>
<td>$&gt;1.8 \times 10^{-4}$</td>
<td>21</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The reaction pathway of milacemide oxidation by MAO-B

Milacemide and similar compounds can act as both 'suicide' substrate and specific MAO-B inhibitor.

\[
\begin{align*}
\text{C}_6\text{H}_{12} & - \text{NH} - \text{CH}_2 - \text{C} \quad \text{MILACEMIDE} \\
\text{FAD} & \\
\text{FADH}_2 & \\
\text{C}_6\text{H}_{12} - \text{CH} = \text{N} - \text{CH}_2 - \text{C} & \quad \text{(Putative intermediate inhibitor)} \\
\text{O}_2, \text{H}_2\text{O} & \\
\text{H}_2\text{O}_2 & \\
\text{Pentanoic acid} + \text{NH}_2 - \text{CH}_2 - \text{C} & \quad \text{GLYCINAMIDE} \\
\text{CO}_2 + \text{H}_2\text{O} & \\
\text{NH}_2 & \quad \text{GLYCINE}
\end{align*}
\]

behaves in a manner similar to that of MPTP as a 'suicide substrate' [14]. Indeed, in vitro, after single or repeated oral administration (6 months, 300 mg/kg), a specific inhibition of MAO-B is apparent in the brain and liver [13, 15]. However, in contrast to the irreversible inactivation of MAO-B by 1-deprenyl, in vivo, the recovery of this enzyme activity after milacemide is significantly faster, suggesting a partially reversible inactivating property. Nevertheless, this action does not preclude the ability of milacemide to inhibit MAO-B specifically and increase brain levels of phenylethylamine and dopamine in two species of monkeys (Cynomolgus and Rhesus monkeys) [15], with the concomitant increases of dopamine metabolites, homovanillic acid and dihydrophenylacetic acid [15] in the striatum. This finding would indicate that milacemide could be used in the therapy of Parkinson's disease in the same way as 1-deprenyl [16, 17] and may also have a neuroprotective profile [18-20], like the latter compound.

The ability of glycine to cross the BBB as an aminoacemamide led us to synthesize a large number of aliphatic and aromatic amine derivatives of glycine and GABA. Tables 1 illustrates five such compounds. With the exception of Compound 1, all compounds (2-5) examined have a greater affinity and \( V_{\text{max}} \) for MAO-B than for MAO-A. Compound 1 (2-n-pentylaminodiacetamide) was oxidized equally by MAO-A and B. As a whole the GABA derivatives are poorer substrates for both enzyme forms, although they have a slightly greater affinity for the B enzyme. Like milacemide, compounds 1 and 2 are enzyme-activated, partially-reversible specific inhibitors of MAO-B in vitro and in vivo. Their in vitro inhibitory activity on amine metabolism have not been examined; however, preliminary studies in rats do indicate that they possess anti-convulsant activity in buccuculin- and picrotoxin-induced convulsion. It remains to be determined whether the latter property is associated with their metabolites.

The phenomenon that brain-impenetrable amino acids (e.g. glycine) can be made to pass the BBB, when coupled to a non-polar MAO-B substrate (e.g. pentyamine and phenylethylamine) is an important concept. This process could be an extremely useful procedure by which other drugs can be transported across the BBB.

### Other endogenous MAO-B substrates

Dopamine and phenylethylamine are recognized as the main pharmacologically active substrates of MAO-B [1, 2]. Both amines have been linked to the anti-Parkinson action of the selective MAO-B inhibitor l-deprenyl [16-18]. Brains obtained at autopsy from l-deprenyl-treated Parkinson's disease subjects have shown that both dopamine and phenylethylamine are increased in extrapyramidal regions (caudate nucleus, substantia nigra, putamen and globus pallidus). However, the increase (>3500%) in phenylethylamine is substantially greater than that of dopamine which can also be oxidized by MAO-A [17]. This has led to the suggestion [20] that phenylethylamine could act as a sustained modulator of dopamine release, because of its amphetamine-like action.

Besides its ability to potentiate the pharmacological action of dopamine formed from l-dopa [16], l-deprenyl is thought to have neuroprotective activity in nigro-striatal dopamine neurons in Parkinson's disease [18]. The recent confirmation of this hypothesis [19, 21] does not allow any conclusion about the mechanisms of the possible neuroprotective action of l-deprenyl to be drawn. This mechanism may not necessarily depend on the inhibition of oxygen free radicals formed from \( \text{H}_2\text{O}_2 \) generated by MAO-B reaction [22]. The polyamines putrescine, spermine and spermidine, whose concentration in brain astrocytes can reach
Acetylated derivatives of polyamine act as potent activators of ODC [23-25]. In the absence of an active polyamine oxidase enzyme in the brain these amines are N-acetylated. The latter derivatives are now thought to be selective substrates of MAO-B. Acetylated derivatives of polyamine act as potent activators of ODC [23-25]. In the absence of an active polyamine oxidase enzyme in the brain these amines are N-acetylated. The latter derivatives are now thought to be selective substrates of MAO-B.

![Diagram of polyamine metabolism](image-url)

**Fig. 2**

The pathway of polyamine metabolism via conversion of ornithine by ornithine decarboxylase (ODC) to putrescine, spermidine and spermine.

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**Amine Oxidases**

micromolar concentrations, are thought to play an important role in neuronal growth differentiation, plasticity and viability [23]. In the absence of an active polyamine oxidase or diamine oxidase in the brain, these amines are thought to be oxidized by MAO after N-acetylation [23, 25]. Indeed Seiler & Therib [26] showed that N-acetylputrescine was a substrate of MAO in vitro. Treatment of rats in vivo with the MAO inhibitor, iproniazid, resulted in increased brain accumulation of N-acetylputrescine, which is normally present at extremely low concentrations in the brain. Recently we have shown that not only N-acetylputrescine but N-acetyl spermidine and N-acetyl spermine are selective substrates of MAO-B [2] with relative $K_m$ values of 59, 110 and 82 $\mu M$. This finding may have a far greater importance than previously considered. N-Acetylated polyamines exert a profound positive feedback stimulation on ornithine decarboxylase activity, the rate-limiting enzyme in the synthesis of polyamines (Fig. 2). Thus they are thought to have an important regulatory role in the synthesis of parent polyamines. The neuroprotective roles of the polyamines and their N-acetylated derivatives in neurotoxin (MPTP or 6-hydroxydopamine) induced nigrostriatal dopamine neuron degeneration requires investigation. Furthermore an investigation of the concentrations of these polyamine derivatives in extrapyramidal brain regions of idio-pathic Parkinsonism, as well as in 1-depenyl-treated subjects would contribute to the delineation of their function in Parkinson's disease.

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Properties and functions of the semicarbazide-sensitive amine oxidases

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The semicarbazide-sensitive amine oxidases (SSAOs) are grouped together under the Enzyme Commission classification, EC 1.4.3.6 [amine: oxygen oxidoreductase (deaminating) (copper containing)] and have a history dating back to the discovery of histaminase by Best in 1929 [1]. This enzyme was subsequently renamed diamine oxidase (DAO), to help establish its separate identity from monoamine oxidase (EC 1.4.3.4; MAO). Doubts about this terminology were expressed when it was shown that some long chain diamines were substrates for MAO and not DAO, while other enzyme activities capable of deaminating mono-, di- and polyamines were beginning to appear (see [2] for review). This led Blaschko [3] to introduce terminology based on the sensitivity of a particular amine oxidase to carbonyl reagents such as semicarbazide and hydroxylamine. One group contained 'enzymes resistant to inhibition by carbonyl groups' and included the classical MAO enzymes as well as a number of other FAD-dependent enzymes. The other group contained 'enzymes inhibited by carbonyl reagents'. Its members included monoamine oxidases and enzymes in plants and bacteria as well as enzymes in blood plasma that were capable of deaminating monoamines such as benzylamine and ruminant plasma enzymes that deaminate the polyamines, spermine and spermidine [2, 4]. Zeller [5] proposed that 'carbonyl reagent' should be replaced by 'semicarbazide' since some carbonyl reagents were also active against the first group of enzymes while semicarbazide was not. Since the introduction of this classification, a number of additions have been made and the name SSAO has gained in acceptance. Such additions include lysyl oxidase (protein-l-lysine 6-oxidase; EC 1.4.3.13) [6] and an assortment of tissue-bound amine oxidases found mainly in brown and white fat as well as smooth muscles, especially those of blood vessels. The SSAO group appears to grow inexorably and, as yet, the great majority of its constituent enzymes have no rigorously defined name. Several trivial names have been used to describe both these tissue-bound and the plasma enzymes, including benzylamine oxidase and clorgyline-resistant amine oxidase [4]. However, SSAO appears to be the most suitable name at present since the criterion for an enzyme to be included in this group is that its ability to catalyse oxidative deamination is inhibited by semicarbazide. No other characteristic is required for inclusion.

SSAO, like MAO, catalyses a double-displacement, or ping-pong reaction, although it would appear to be of the aminotransferase type:

\[ E-\text{CHO} + R-\text{CH}_2-\text{NH}_2 \rightarrow E-\text{CH}_2-\text{NH}_2 + R-\text{CHO}\]

In such a reaction, release of ammonia is dependent upon \(O_2\), whereas MAO can release \(\text{NH}_3\) anaerobically. This, along with studies on the kinetics of inhibition of product formation, favour the aminotransferase pathway [7, 8].

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Abbreviations used: AA, allylamine (3-aminopropene); ACR, acrolein (prop-1-en-3-one); B24, 3,5-ethoxy-4-aminomethylpyridine, HCl; DAO, diamine oxidase; EDRF, endothelium-derived relaxing factor; MAO, monoamine oxidase; MDL 72145, (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine; PCZ, N-isopropyl-alpha-(2-methylhydrazino)-p-toluamide; HCl (procarbazine); PQQ, pyrroloquinoline quinone; SSAO, semicarbazide-sensitive amine oxidase; GSH, reduced glutathione.

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