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Perspectives on MAO-B in Aging and Neurological Disease

Where Do We Go From Here?

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Abstract

The catecholamine-oxidizing enzyme monoamine oxidase-B (MAO-B) has been hypothesized to be an important determining factor in the etiology of both normal aging and age-related neurological disorders such as Parkinson's disease (PD). Catalysis of substrate by the enzyme produces H_2O_2 which is a primary originator of oxidative stress which in turn can lead to cellular damage. MAO-B increases with age as does predisposition towards PD which has also been linked to increased oxidative stress. Inhibition of MAO-B, along with supplementation of lost dopamine via L-DOPA, is one of the major antiparkinsonian therapies currently in use. In this review, we address several factors contributing to a possible role for MAO-B in normal brain aging and neurological disease and also discuss the use of MAO-B inhibitors as drug therapy for these conditions.

Index Entries: Monoamine oxidase B; Parkinson's disease; aging; free radicals; deprenyl; genetic polymorphisms; mitochondrial dysfunction.

Introduction

The isolation of monoamine oxidase (MAO; E.C. 1.4.3.4) in 1928 by Hare and the subsequent characterization of its localization, substrate specificity, inhibition, and biochemistry have been eloquently described in several reviews (e.g., refs. *1,2*). MAO is an integral protein of the outer mitochondrial membrane and occurs in both neuronal and non-neuronal cells in the brain as well as in peripheral organs. It is the primary enzyme in the brain involved in degradation of biogenic amines such as catecholamines *(3).* Oxidation of amines from both endogenous and exogenous sources by MAO influences the concentration of neurotransmitter amines as well as that of many xenobiotics *(4,5).* As such, MAO has become a primary molecular target for therapeutic drug development *(6).*

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MAO can be classified into two isoforms, A and B, according to inhibitor sensitivity and substrate specificity. MAO-A and MAO-B are encoded by separate genes that are closely linked on the X chromosome and the resulting proteins share 70% similarity in amino acid sequence *(7–9).* Both isoforms are found in high levels in the brain: MAO-A predominantly in catecholaminergic neurons and MAO-B primarily in non-neuronal cells such as astrocytes and radial glia, but also in serotoninergic neurons *(10–12).* While MAO-B preferentially breaks down the trace amine phenethylamine (PEA) and is selectively inhibited by L-deprenyl *(13),* MAO-A preferentially oxidizes norephinephrine and serotonin and is selectively inhibited by clorgyline *(14).* Both forms oxidize dopamine, tyramine, and octopamine *(15).* Dopamine oxidation is accompanied stoichiometrically by the reduction of oxygen to hydrogen peroxide *(16,17).*

Although the promoter sequences of both genes share approx 60% identity *(18),* the regulation of MAO-A and -B activities are differentially governed at a transcriptional level by specific transcription factors. The human MAO-B promoter has been cloned and characterized and several fragments have been investigated via reporter gene assays *(18,19).* The transcription factor Sp1 and various other members of the Sp family have been shown to be important for the regulation of this isoform of the gene *(20).* The expression of MAO-B induced by Sp1 family transcription factors is mediated by the activation of protein kinase C and MAPK signaling pathways via c-Jun and Egr-1, by a decreased Sp3/Sp1 ratio, and reduced DNA methylation *(20–22).* The DNA-binding activity of the transcriptional regulator Sp1 displays an age-dependent decline *(23,24)* and the transcription factor Egr-1 is a major regulator of cell senescence during replication *(25).* The potential for age-related changes in these transcription factors to impact on MAO-B expression is of interest especially in light of the fact that the aging process itself appears to have a profound effect on levels of the enzyme.

Potential Involvement of MAO-B in Brain Aging and Parkinson's Disease

Previous enzymatic assays performed on postmortem human brain tissue suggest that MAO-B levels increase with age. In the human brain, increased MAO-B activity was found to be the result of an increase in both enzyme concentration and quantity. In contrast, no consistent change in MAO-A activity has been reported with either age *(26–29)* or neurodegenerative disease *(30)* although there are a few exceptions *(31).* It has been postulated that the observed two- to threefold age-related increase in brain MAO-B activity may contribute to cellular degeneration in the brain owing to corresponding increases in the production of hydrogen peroxide as a byproduct of amine oxidation by the enzyme *(30,32).* Hydrogen peroxide produced by MAO-B can in turn be converted into highly toxic hydroxyl radicals, particularly through interaction with iron via the Fenton reaction. Hydroxyl radicals produced owing to increased levels of oxidized glutathione, hydrogen peroxide, and superoxide can extract methylene hydrogens from polyunsaturated fats in neural membrane phospholipids, initiating lipid peroxidation and cell death. They can also nick DNA and damage essential cellular enzymes and structural proteins *(33).* Since MAO-B is an integral protein of the outer mitochondrial membrane, MAO-B catalyzed increases in free radical production may therefore also help explain the age-related increased incidence of mitochondrial DNA damage in the brain *(34).*

Levels of MAO-B appear to be highest in the brain in the thalamus, striatum, cortex, and brainstem *(35,36).* The substantia nigra (SN) contains a large number of MAO-B-positive astrocytes and this may contribute to oxidative stress in this brain region *(11,12).* Although MAO-B is expressed primarily in glial and not in dopaminergic cells, hydrogen peroxide has a high membrane permeability and therefore it can induce toxic effects not only within the cell

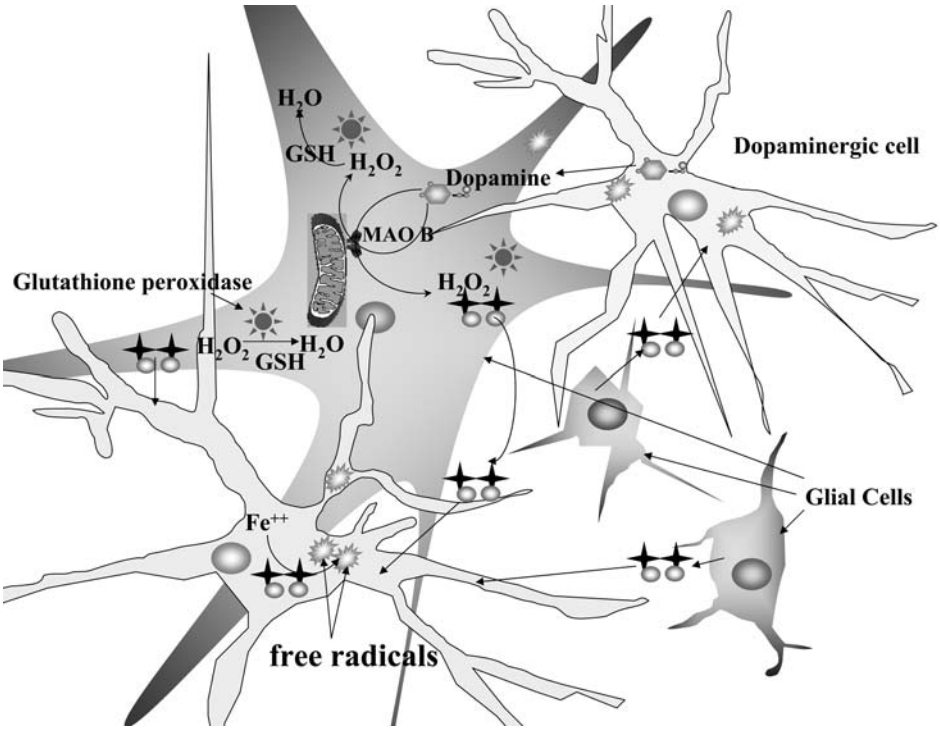

Fig. 1. MAO-B-mediated H_2O_2 production in glia may be deleterious to nearby dopaminergic neurons. MAO-B located in glial cells in the SN can deaminate dopamine and other substrates to form H_2O_2. H_2O_2 is highly membrane-permeable and is able to cross into neighboring dopaminergic cells where it may react with free iron (Fe^{++}) to produce toxic hydroxyl radical which can damage cellular components such as proteins, lipids, and DNA. The glial cells themselves are protected from toxic levels of H_2O_2 by possessing high levels of glutathione and glutathione peroxidase which act to detoxify H_2O_2 to water.

of origin, but also in neighboring cells (Fig. 1) *(37).* Glial cells themselves are somewhat protected against the effects of hydrogen peroxide as a result of the fact that they contain high levels of both glutathione and glutathione peroxidase which act in concert to detoxify hydrogen peroxide within cells by reducing it to H_2O *(38–41).* In fact, the glutathione system is the primary system in the brain for removal of hydroperoxides *(42).* Neurons, which contain significantly lower levels of these protective components, are particularly vulnerable to this mild oxidizing agent *(43–45).* This suggests that hydrogen peroxide produced within glial cells by MAO-B is either broken down to H_2O within these cells by the glutathione system or diffuses into vulnerable nearby neurons *(46).* Oxidative stress may thus act as a predisposing or precipitating factor in the vulnerability of the brain especially the SN to age-related neurodegenerative diseases such as Parkinson's disease (PD) which involves degeneration of dopaminergic neurons in this particular brain region *(12,47–50).* Free iron levels have also been found to be elevated in the SN of severely affected PD patients, which could act along with increased local glial MAO-B levels to cause increased free radical production and subsequent cell damage to dopaminergic neurons *(51).* Increased levels of both lipid peroxidation and 8-hydroxy-2-deoxyguanosine respectively, are indicators of increased oxidative membrane and DNA damage and have been documented in the SN of PD patients

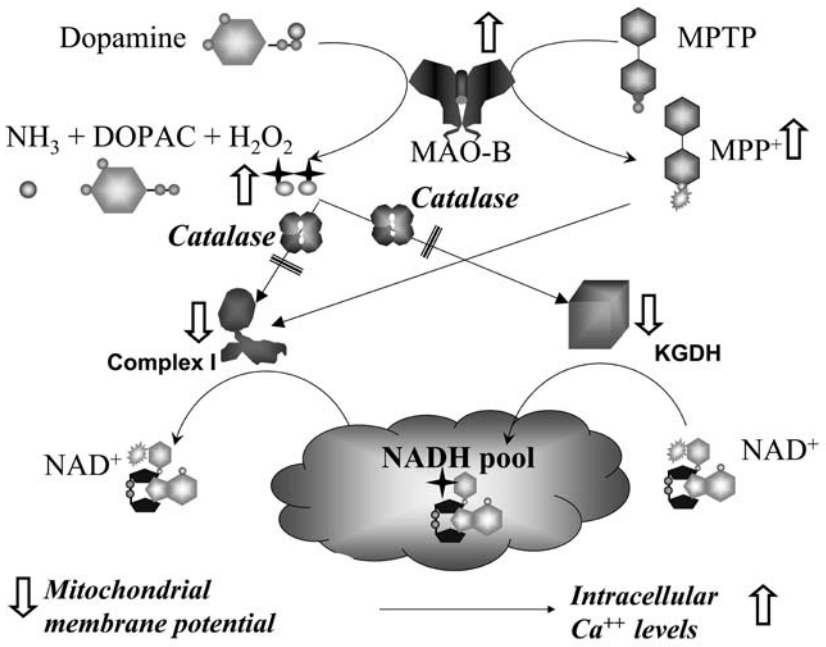

Fig. 2. Possible role of MAO-B elevation in mitochondrial dysfunction. Age-related elevations in MAO-B theoretically could result in both increased H_2O_2 production owing to increased substrate metabolism and increased production of MPP^+-like entities via metabolism of exogenous or endogenous substrates related to MPTP. Both H_2O_2 and MPP^+ may in turn exert a direct inhibitory influence on complex I. H_2O_2 produced via elevated MAO-B may also inhibit KGDH activity levels. Direct inhibition of complex I may impact directly on mitochondrial respiration whereas decreases in KGDH activity could contribute to decreased respiratory capacity. Ultimately, these direct and indirect effects on complex I may lead to increased sensitivity to stress-mediated mitochondrial dysfunction.

compared with normal controls *(52).* It could be hypothesized that an initial PD-related loss of dopaminergic SN neurons results in injury-related gliosis producing an increase in hydrogen peroxide generation via increased MAO-B levels to an extent that the glutathione system is overwhelmed, resulting in increased oxidative stress and subsequent death of further vulnerable neurons in the area.

PD has also been associated with decreased mitochondrial function owing to a selective inhibition of mitochondrial complex I activity and the TCA cycle enzyme alpha-ketoglutarate dehydrogenase (KGDH) *(53–57).* Age-related increases in mitochondrial damage may be caused in part by increased MAO-B levels and subsequent oxidative stress that could decrease mitochondrial respiration to a threshold which, upon further inhibition by other age-related events, could reduce it to a level that can no longer accommodate cellular needs. Dopaminergic neurons such as those of the SN are particularly susceptible to mitochondrial inhibition as they have high energy requirements *(58,59).* We have recently demonstrated that elevation of MAO-B levels mimicking normal age-related increases in the enzyme results in a dramatic inhibition of both mitochondrial complex I and KGDH activities in cultured dopaminergic cells via increased H_2O_2 production by MAO-B (Fig. 2) *(60).* Importantly, findings from our study further demonstrate that KGDH inhibition by MAO-B generated oxidative stress is likely to be of more importance

than direct inhibition of complex I activity itself in defining the maximal respiratory capacity of the mitochondria. This suggests that preservation of KGDH activity is of primary importance in maintaining spare respiratory capacity in dopaminergic mitochondria and as such has major implications for PD *(60).*

Impact of Genetic Differences in MAO-B on Vulnerability to PD

Genetic polymorphisms in the *MAO-B* gene relevant to PD etiology and pathogenesis have been extensively researched because of their potential role in the disease. Interestingly, a study of variations in the GT nucleotide repeats in intron 2 of the gene showed a significant difference in allele frequencies between PD cases and controls only in a subgroup of older subjects *(61).* A specific G to A transition polymorphism in intron 13 of the gene detected by single-strand conformational polymorphism analysis *(62–64)* has also been reported to be associated with a twofold increase in risk for PD in the Caucasian population *(65).* This allele may interact with other genetic loci such as particular alleles of the NAD(P)H:quinone oxidoreductase *(NQO1)* or catechol-*O*-methyltransferase *(COMT)* genes in a synergestic manner resulting in an increase PD incidence in individuals as well as with various environmental factors *(66–68).*

Sex Differences in PD and Their Relationship to MAO-B

In addition, there is a strong gender difference with respect to PD incidence: it is found to be more prevalent in males than in females and the progression of the disease to be more rapid. Although the *MAO-B* gene is located on the X chromosome, it is not believed that this plays a role in these noted sex differences. Rather, the female sex hormone estrogen has been suggested to have modulatory effects in PD, although it is unclear whether these are truly neuroprotective or simply symptomatic (reviewed in *69–71*). Sexual differences have however been reported in regards to the modifying effect of the intron 13 A/G MAO-B allelic genotype and a noted inverse correlation between smoking and PD *(72).*

Use of MAO-B Inhibitors as Drug Therapy for PD

In 1983, it was reported that 1-methyl-4-phenyl-1,2,3,5- tetrahydropyridine (MPTP), an impurity in a street drug, caused an initially puzzling outbreak of PD in a number of young people *(73,74).* Administration of this protoxin to primates and other mammals results in specific damage to the nigrostriatal dopaminergic system mimicking that seen in PD. It was discovered that MAO-B inhibition prevented MPTP-induced neurotoxicity by inhibiting the conversion of MPTP to 1-methyl-4-phenylpyridinium (MPP^+), which is toxic to dopamine neurons *(75).* MPP^+, which is taken up by a receptor-mediated system into dopaminergic neurons, has been suggested to act by inhibiting mitochondrial complex I and via production of oxidative stress *(76,77).* The selective effects of MPTP on this brain region led to the hypothesis that PD may be associated with an MAO-B-catalyzed event.

MAO-B is specifically and irreversibly inhibited by L-deprenyl (known medically as selegiline) and, as such, this drug is currently an important member of the repertoire of drugs currently used as therapy for PD *(78,79).* Deprenyl is structurally related to the MAO-B specific substrate PEA; it acts by binding the flarin adenine dinucleotide (FAD) domain and therefore blocks oxidation of substrate *(80).* Previous studies suggest that deprenyl protects not only against cell death associated with cellular and animal models of PD *(81,82),* but also may delay progression of the disease itself *(83).* As a monotherapy in early PD, deprenyl provides a mild antiparkinsonian effect. As first demonstrated by Birkmayer et al., when given concomitantly with the dopamine precursor

L-DOPA it potentiates L-DOPA's effects *(84,85)* and allows a reduction in daily L-DOPA dosage *(86).* In patients with fluctuating responses, deprenyl decreases their severity *(87).*

In patients, deprenyl is selective for MAO-B at a dose of 10 mg/d but its pharmacokinetics are highly variable *(88).* After oral administration of a single dose, plasma deprenyl levels reach a peak concentration of 2 μg/L in under 1 h. Platelet MAO activity is reduced to less than 20% of normal levels and the half-life of its recovery after drug removal is around 2 wk. Deprenyl's elimination half-life in the urine is around 1–2 h which appears to be via an extrahepatic mechanism since it is more rapid than liver blood flow. Its amphetamine-related metabolites, which themselves have neuronal activity, have longer half-lives ranging from 4–20 h. Transdermal administration of selegiline results in an increase in plasma concentrations of the drug and a decrease in the formation of its metabolites, suggesting that this is a superior route to oral administration.

After daily administration for a period of a week, both selegiline itself and its metabolites accumulate in tissues including the brain resulting in a one- to fourfold increase in their half-life compared to plasma levels *(89).* Selegiline is able to enter the brain due to its high lipid solubility. However, only approx 20% of central nervous system MAO appears to be inhibited by selegiline. Its half-life of recovery in the brain is approx 40 d. Owing to its varied pharmokinetics, low bioavailability, and extensive distribution, deprenyl may not be the most ideal agent for elicitation of MAO-B inhibition in context with PD.

Interpretation of drug studies with deprenyl are also complicated by the fact that in addition to acting as a direct inhibitor of MAO-B, deprenyl has also been suggested to have antioxidant properties including upregulation of activities of antioxidant enzymes such as superoxide dismutase and catalase in brain dopaminergic regions *(35,90–92).* Moreover, deprenyl is able to suppress the formation of and scavenge hydroxyl radicals in vivo *(81).* In the SN, chronic administration of deprenyl to aging rats produced a decrease in free radical-mediated protein oxidation as measured by a decrease in carbonyl groups compared with untreated controls *(93).* The pro-apoptotic protein c-Jun is also decreased by deprenyl and other propargylamines, indicating a possible transcriptional role for the pharmacological MAO-B inhibitor *(94).* It is unclear whether the observed prevention of disease-related changes are attributable to the direct inhibition of MAO-B itself or to other deprenyl-related effects.

A recent report indicates that D-deprenyl, the less active isomer of L-deprenyl, is also neuroprotective *(95).* Other selective MAO-B inhibitors are currently being examined for their ability to slow the clinical progression of PD with the hopes that this may help overcome some of the difficulties inherent with deprenyl itself. For example, rasagiline, a potent irreversible MAO-B inhibitor *(96),* is currently under clinical trials for PD *(97)* as well as lazabemide, a reversible inhibitor of the enzyme *(98),* neither of which are metabolized into active compounds in the same manner as deprenyl *(99).* Again, however, the noted neuroprotection achieved via use of these compounds may be attributable to properties other than MAO-B inhibition such as reported neurotrophic or antipoptotic effects *(100,101).*

Some clues as to potential novel inhibitors have come from surprising sources. Cigarette smokers have been shown to have reduced MAO-B activity levels as a result of a pharmacological effect of tobacco smoke exposure on the enzyme *(2,26,102,103)* putting them at a lower risk for parkinsonism. This inhibition has been suggested to be mediated by two components identified in cigarette smoke: 2,3,6-trimethyl-benzoquinone *(104)* and 2-naphthylamine *(105),* which appear to have inhibitory effects against MPTP-induced toxicity in mice *(106).* 1,2,3,4-Tetrahydroisoquinoline, a proneurotoxin that has been suggested by some researchers to be involved in the development of PD by a mechanism similar to that of MPTP, forms cyanoamines with components of cigarette smoke which inhibit MAO-B

(107). These findings provide support to the thesis that components of tobacco smoke may be responsible for the inhibition of MAO-B in human smokers. Further research in this area may uncover novel MAO-B inhibitors to test as therapies for the disease.

Potential Role of MAO-B in Other Neurological Conditions

Deprenyl treatment not only slows the disabling progression of PD, but also appears to retard cognitive decline in Alzheimer's disease (AD) in terms of both improvement in verbal memory performance and increased lag time between diagnosis and nursing home placement *(108–111).* It is unclear whether these effects are attributable to the inhibition of MAO-B or to other properties of the drug. However, MAO-B activity has been found to be increased at early disease stages in the frontal cortex of AD patients vs age-matched controls and the order of magnitude of the MAO-B increase appears to correlate with disease severity *(112–116).* This increase could be ascribed to increased gliosis However, levels of platelet MAO-B, a peripheral marker of MAO-B activity, are also increased in AD patients *(117).* MAO-B activity is found to be present at high levels in astrocytes associated with senile plaques in the hippocampus and cortex of AD brains *(118).* In addition, dopamine levels have been found to be more severely decreased with age in the cortex and hippocampus of AD patients compared to age-matched controls; deprenyl treatment results in lower levels of the dopamine metabolite HVA in the cerebral spinal fluid (CSF) of treated patients *(119–125).* Microapplication of deprenyl to monkey forebrain and recordings of single-neuron activity demonstrated that the drug has effects on catecholaminergic-sensitive neurons in this brain region *(126).*

In addition to its putative role in neurodegenerative disease, MAO-B may be involved in acute brain injury as well. For instance, several reports suggest that various MAO-B inhibitors prevent the generation of hydroxyl radicals and cerebral injury normally elicited as a consequence of ischemia/reperfusion in animal models as well as stroke patients *(127–129)* although contradictory results have been reported *(130).* However, absence of the MAO-B gene has been found to be nonprotective and, in fact, detrimental in an animal model of acute cortical infarction *(131).*

Conclusions

Based on a growing plethora of evidence, age-related increases in MAO-B activity in the brain are hypothesized to play a major role in both normal aging and age-related neurological disease, particularly PD. Interpretation of the effects of the current class of MAO-B inhibitors, however, is complicated by their MAO-B independent effects. Given this, further research into the mechanism(s) by which genetic alterations in the protein itself may impact on the aging brain and age-related neurological disease as well as development of novel specific inhibitors, are warranted.

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