Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
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4 Amine Oxidases and the Metabolism of Xenobiotics

Keith F. Tipton[1] **and Margherita Strolin Benedetti**[2]

[1] *Trinity College, Dublin, Ireland*
[2] *UCB Pharma, Nanterre Cedex, France*

Introduction

THE AMINE OXIDASE GROUP OF ENZYMES

Mammalian systems contain several different amine oxidases that may be distinguished by their cofactor requirements, substrate specificities and inhibitor sensitivities. The enzymes that are active towards monoamines have been the most extensively studied in terms of their possible involvement in xenobiotic metabolism and these enzymes will be considered first. The monoamine oxidases (amine: oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4), which are flavoproteins (FAD), are sensitive to inhibition by acetylenic-amine derivatives such as clorgyline, deprenyl (selegiline) and pargyline but are not inhibited by carbonyl-group reagents such as semicarbazide. The so-called semicarbazide-sensitive amine oxidases (amine: oxygen oxidoreductase (deaminating) (copper-containing); EC 1.4.3.6) contain, in addition to copper, a carbonyl-type group at their active sites. This is now believed to be peptide-bound 3,4,6-trihydroxyphenylethylamine (TOPA) in its quinone form. Theses enzymes are inhibited by semicarbazide but not by the acetylenic monoamine oxidase inhibitors. Both these groups of enzymes catalyse the oxidation of amines according to the overall reaction:

$$RCH_2NR'R'' + O_2 + H_2O \longrightarrow RCHO + NHR'R'' + H_2O_2$$

Although they are involved in the metabolism of some xenobiotics, the products of the reaction, ammonia, hydrogen peroxide and an aldehyde are themselves potentially toxic. The aldehydes formed may be further metabolised by the aldehyde dehydrogenases or aldehyde oxidases to the corresponding carboxylic acids or by the aldehyde reductases to the alcohols, as shown in Figure 4.1.

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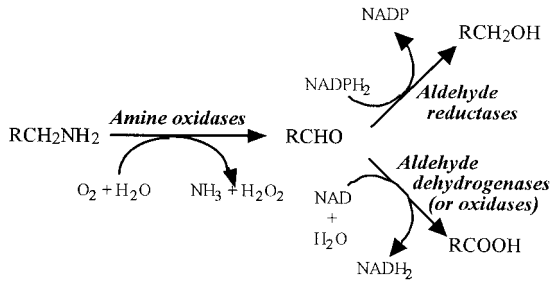


Figure 4.1 General outline scheme of amine metabolism through amine oxidases.

Since aldehydes can be quite reactive and rather difficult to detect, their existence as immediate products of xenobiotic metabolism has, in many cases, been inferred rather than directly demonstrated. Furthermore, some deductions about the involvement of amine oxidases in the metabolism of xenobiotics have been based on little more than the demonstration of the formation of the corresponding carboxylic acid or alcohol. However, since some microsomal monooxgenases can catalyse the conversion of amines to aldehydes, this cannot be regarded as compelling evidence. The fact that some amine oxidase inhibitors also affect cytochrome P-450-dependent monooxygenases (Dupont *et al.* 1987), also means that inhibitor studies should be evaluated to take account of this possibility.

As more is known about the involvements and diverse specificities of the monoamine oxidases, these will be considered first.

The monoamine oxidases

The properties and behaviour of the monoamine oxidases have been extensively reviewed (Abell and Kwan 2000; Shih *et al.* 1999; O'Brien and Tipton 1994; Wouters 1998) and this account will be restricted to factors that may affect, or be affected by, their interaction with xenobiotics.

STRUCTURE OF THE MONOAMINE OXIDASES

The monoamine oxidases oxidise aliphatic and aromatic primary amines and some secondary and tertiary amines. Two isoenzymes of monoamine oxidase, MAO-A and MAO-B, are present in most mammalian tissues. These enzymes were originally distinguished by their sensitivities to inhibition by the acetylenic inhibitors clorgyline and deprenyl and by their substrate specificities. They are now known to be encoded by separate genes, which are both located on chromosome X.

The two enzymes share a relatively high degree of sequence identity. Comparison of the sequences of the different isoenzymes among species shows that there is a greater degree of similarity between the same isoenzyme from different species than between different isoenzymes from the same species. For example, ox and human MAO-B have

approximately 90% of amino acid residues identical whereas the overall identity of amino acid sequences for MAO-A and MAO-B from the same species is about 70%. There are discrete and scattered differences in amino acid sequences between ox MAO-A and MAO-B and human MAO-A and MAO-B. The high degree of sequence identity suggests that the genes for MAO-A and MAO-B are derived from a common progenitor gene. Despite the relatively high level of sequence identity between MAO-A and MAO-B from different species, there are species differences between their interactions with some substrates and inhibitors which indicated that it is unwise to attempt to extrapolate from the xenobiotic metabolising activities in experimental animals to the human situation.

SUBSTRATE AND INHIBITOR SPECIFICITIES

Some preferred substrates and inhibitors are shown in Figures 4.2 and 4.3. Typically MAO-A is inhibited by low concentrations of clorgyline and catalyses the oxidative deamination of 5-hydroxytryptamine (5-HT) whereas MAO-B is inhibited by low concentrations of (-)-deprenyl (selegiline) and is active towards benzylamine and 2-phenylethylamine (PEA). These substrate specificities are not absolute. For example, 5-HT is a substrate for MAO-B as well as MAO-A in rat brain and liver. However, in the rat brain the K_m and V_{max} values for MAO-B are six-fold higher and 9-fold lower, respectively, than those for MAO-A. Hence, relatively low concentrations of 5-HT will be oxidised essentially by MAO-A alone, whereas at very high concentrations the MAO-B form could contribute about 10% of the total activity. Similarly, 2-phenylethylamine (PEA) is a substrate for MAO-A but with a considerably higher K_m, and a V_{max} value that is 5-6-fold lower than the corresponding values for MAO-B. Thus, although the A form can contribute to the total activity at very high concentrations of PEA, only the activity of the B form is important at low concentrations of this substrate. In rat heart, which contains very little MAO-B, it has been reported to be possible to detect low levels of benzylamine oxidation by MAO-A. Such factors may be important in understanding the relative contributions of different tissues to the metabolism of xenobiotics.

In most species examined, tyramine is a substrate for both enzymes, as are dopamine and noradrenaline in the human brain. Although tyramine has been frequently regarded as a substrate for both forms of MAO, the kinetic parameters towards this substrate differ. With the enzymes from rat liver mitochondria, for example, the K_m and V_{max} values of MAO-A towards this substrate were 107 μM and 11nmol.min^{-1}.mg protein^{-1}, respectively, whereas the corresponding values for MAO-B were 579 μM and 20 nmol.min^{-1}.mg protein^{-1}. Therefore, the proportions of the total activity towards tyramine that are contributed by each of the two forms will depend to some extent on the concentration of substrate. Thus about 32% of the total rat liver MAO activity towards 50 μM tyramine will be due to MAO-B, but this proportion will rise to 57% at a tyramine concentration of 1 mM. Hence, there is no simple subdivision into specific MAO-A, specific MAO-B and mixed substrates but rather a continuum.

Figure 4.2 indicates the substrate selectivities of the monoamine oxidases for a range of substrates. These refer to the enzymes from human liver and brain. There

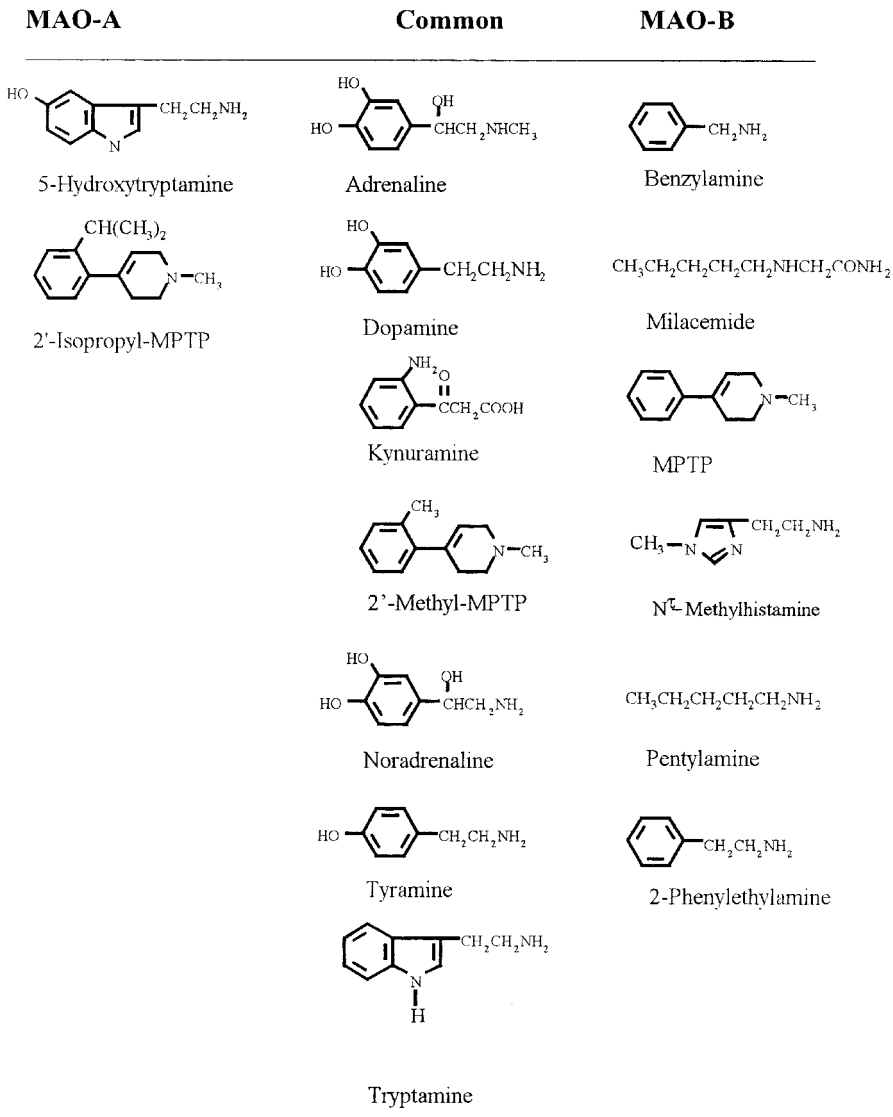


Figure 4.2 Some common monoamine oxidase substrates, arranged according to their preferences for MAO-A or MAO-B or whether they are good substrates for both isoenzymes (common). The classification refers to the monoamine oxidases from human brain and liver; the enzymes from other species may not behave identically.

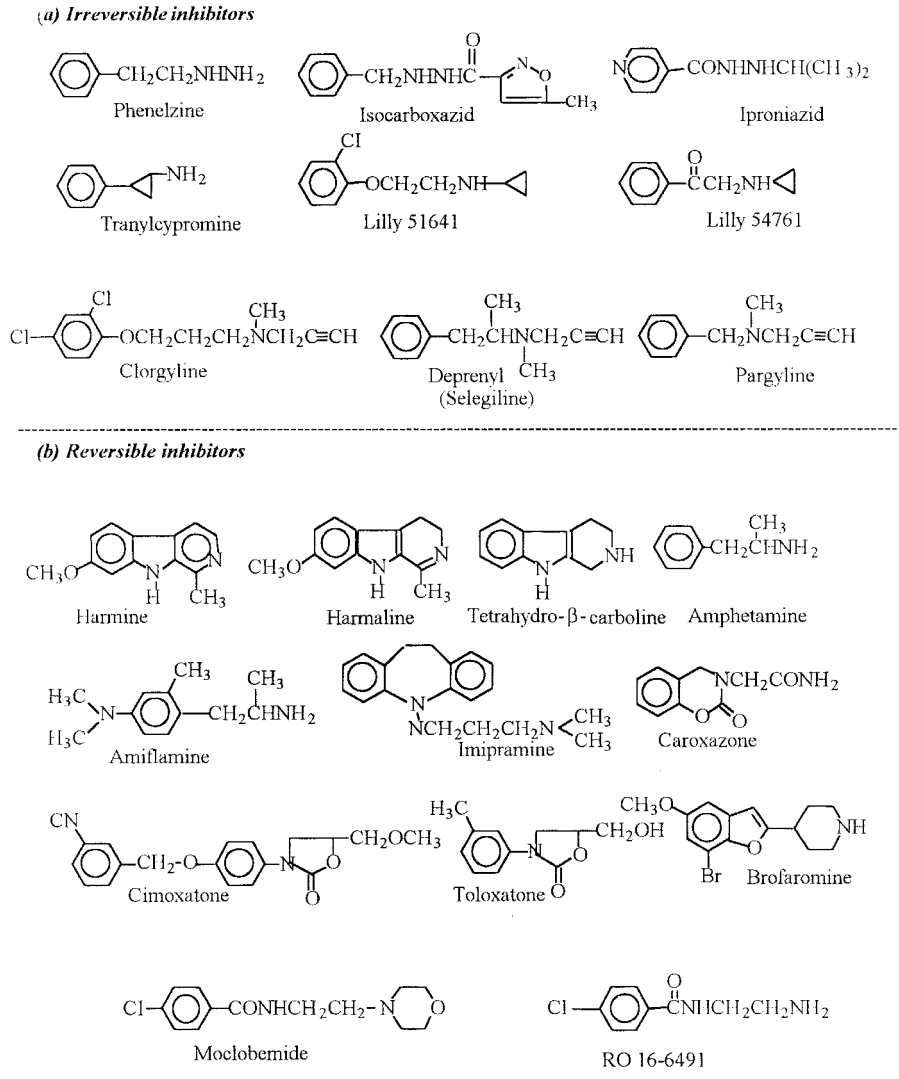


Figure 4.3 Some monoamine oxidase inhibitory compounds.

may, however, be species differences between the specificities of the enzymes and so it cannot be assumed that the behaviour will necessarily be identical in all tissues and species.

The crystal structure of MAO has not yet been determined and, although there have been several attempts at determining structure–activity relationships for substrates and

inhibitors, it is still not possible to predict with confidence whether a given compound will be a substrate for either, or both, of the monoamine oxidases.

LOCATION AND DISTRIBUTION

Both MAO-A and MAO-B are tightly associated with the mitochondrial outer membrane. However, significant levels of both MAO-A and MAO-B activities have been found in the microsomal fractions. The properties of the microsomal enzymes appear to be similar to those of the mitochondrial enzyme. MAO has an almost ubiquitous occurrence in the cells of most mammalian species, the most notable exceptions being erythrocytes. Several tissues from different species express essentially only one form of MAO, for example MAO-A predominates in human placenta, rat spleen and heart whereas MAO-B dominates in human blood platelets, ox liver and kidney and pig liver. The distributions of the two isoenzymes in different human tissues are shown in Figure 4.4.

In the rat peripheral nervous system histochemical studies have shown MAO to be localised in the endothelial cells of the endoneurial vessels, the Schwann cells and the neurones in some unmyelinated axons. Microvessels from the blood-brain barrier are rich in MAO-B activity, where it presumably functions as a component of the blood-brain barrier, but a comparison between six mammalian species showed a twenty-five-fold difference in the amount of enzyme present. The two enzymes are rather evenly distributed in the different regions of human brain. Immunohistochemical and

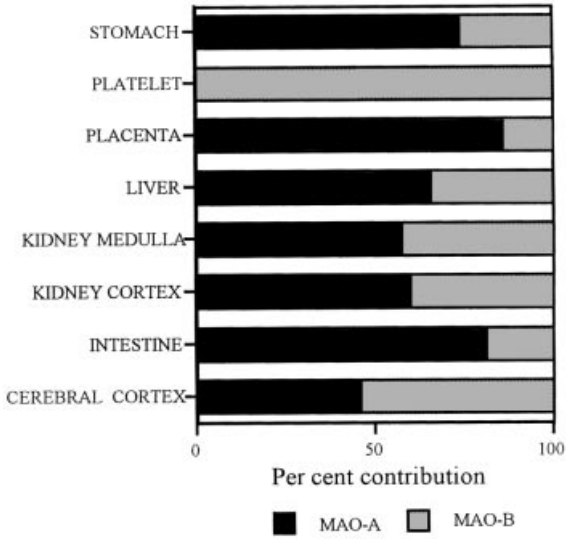


Figure 4.4 The proportions of activities towards tyramine (100 μM) of the two forms of monoamine oxidase (MAO-A and MAO-B) in different human tissues.

pharmacological studies have shown that serotonergic neurones (e.g. cells of the nucleus raphé dorsalis, nucleus centralis superior and glial astrocytes) contain predominantly MAO-B and catecholaminergic neurones (e.g. cells of the substantia nigra, locus coeruleus, nucleus subcoeruleus and the periventricular regions of the hypothalamus) contain predominantly MAO-A. Data obtained with mRNA probes are in general agreement with these results. Positron emission studies in human brain *in vivo*, after intravenous injection of the ^{11}C-labelled inhibitors, clorgyline and (-)-deprenyl showed the anatomical distribution of ^{11}C to parallel the distribution of MAO-A and MAO-B activities in human brain autopsy material.

Relatively high monoamine oxidase activities have also been shown to be present in human liver and kidney (Sullivan *et al*. 1986) and in rat kidney (Squires 1972). These values suggest that MAO may be an important enzyme in first-pass metabolism of amine xenobiotics taken orally. Since only direct administration of amine via an artery will by-pass the lung on the first pass, it is interesting to observe that the activities of MAO-A and MAO-B are significant in that organ. Perfusion studies have shown the lung to be capable of taking up and metabolising amines, particularly those acting as MAO-B substrates such as PEA (Bakhle and Youdim 1979).

REGULATION AND TURNOVER

The levels of MAO-B in blood platelets, and perhaps other tissues, are genetically regulated and appears to reflect personality-type (Garpenstrand *et al.* 2000). MAO-A and MAO-B also differ markedly in their patterns of developmental expression and genetic regulation (Strolin Benedetti *et al.* 1992a), which might affect their xenobiotic-metabolising capacities. In most mammalian species MAO-A appears before MAO-B. Changes in MAO activities during development probably reflects events of cellular differentiation as well as the changing ratios of different cell types within tissues. In the developing rat central nervous system, the increase in MAO-A follows the caudal-rostral pattern of neuronal differentiation. MAO-B activity is at relatively high levels in many tissues at birth, especially the liver. However, the level of this enzyme increases dramatically in the brain after birth probably due to the proliferation of astrocytes.

The process of ageing also appears to involve changes in levels of MAO activity. Increases in human brain MAO-B activity have been observed in several studies, possibly as a result of glial cell proliferation. Results on the effects of age on MAO-A in human brain have been conflicting in that both increases and no change have been reported. In contrast, there is little change in the MAO-A or MAO-B activities in the rat central nervous system from 2 to 28 months of age.

MAO-A, but not MAO-B, activity is elevated in reproductive tissue when levels of progesterone are high. In contrast, oestrogen-treated ovariectomised female rats show increased MAO-B activity and decreased MAO-A activity in the cerebellum and in certain areas of the brain stem. Castration leads to a 3-fold increase in MAO-A activity with no change in the MAO-B activity in humans and this increase can be prevented by administration of oestradiol or testosterone. Thyroxine and adrenal corticosteroids both appear to influence MAO-A and MAO-B levels. Studies with cultured human skin fibroblasts revealed that short (6 h) exposures to several hormones, including

progesterone, testosterone, triiodothyronine and the glucocorticoid, dexamethasone, reduced the total MAO activity in these cells. In contrast, progesterone, testosterone, corticosterone and dexamethasone were found to increase the synthesis of MAO-A, without affecting MAO-B, in capillary endothelial cells.

When MAO is inhibited by an irreversible inhibitor, the rate of recovery of enzyme activity can be used to estimate the rate of turnover of the enzyme in the tissues. This approach has shown the half-lives of MAO-A and MAO-B in rat liver to be similar, in the range 2.5–3.5 days. The half-life in rat brain was found to be about 10–13 days and in heart from the same species the half-life of MAO-A increased with age. The rates of turnover of the two forms of MAO in rat intestine were found to be different, with the half-life values of 2.2 and 7.5 days being reported for MAO-A and MAO-B, respectively. In a study of the turnover of MAO in baboon brain using positron-emission tomography of the deprenyl-labelled enzyme a half-life of about 30 days was determined. Such a slow rate of turnover in the primate brain would have important implications for the 'wash-out' periods used in studies on the effects of irreversible monoamine oxidase inhibitors in the clinical situation. Since either non-selective or MAO-A selective inhibitors have been widely used to treat depression, and MAO-B inhibitors, such as (-)-deprenyl (selegiline), have proven useful in the treatment of Parkinson's disease and may also have value in Alzheimer's disease, their effects must be taken into account in cases where the monoamine oxidases play a significant role in xenobiotic metabolism.

PHYSIOLOGICAL ROLES

The involvement of any enzyme in xenobiotic metabolism might be expected to impair its normal physiological functions through substrate competition. MAO-A and/or MAO-B in peripheral tissues such as the intestine, liver, lung and placenta appear to play a protective role in the body by oxidising amines from blood or preventing their entry into the circulation. Intestinal MAO may be responsible for the inactivation of vasoactive amines such as noradrenaline, tyramine, dopamine and 5-HT of dietary origin. Liver MAO-A and MAO-B may be involved in controlling blood levels of pressor amines, including adrenaline, which have escaped deamination by MAO in the platelets or in the gut. MAO-A in the vascular endothelial cells of the lung is believed to be important in eliminating freely circulating 5-HT from the circulation, thereby protecting the heart and vascular system from the effects of the amine. MAO-A in the syncitial trophoblast layer of the placenta and MAO-B in the microvessels of the blood-brain barrier presumably have similar protective functions since these locations constitute metabolic barriers. Consistent with this are the observations that drugs which inhibit MAO induce extreme sensitivity to the dietary amine tyramine (see below) and infusion of relatively high concentrations of pargyline into amniotic fluid results in abortion.

In the central and peripheral nervous system intraneuronal MAO-A and MAO-B have been suggested to protect neurones from exogenous amines and/or to regulate levels of amines synthesised within a neurone. The low levels of MAO-A immunoreactive material in serotonergic neurones indicates that MAO-A within these neurones might not play a major role in degrading 5-HT (5-hydroxytryptamine; serotonin). The

low affinity of MAO-B for the endogenous transmitter indicates that, rather than limiting cytoplasmic levels of 5-HT, the major role of MAO-B in serotonergic neurones may be to eliminate foreign amines and minimise their access to synaptic vesicles. Hence, MAO-B in serotonergic neurones appears capable of enriching the neuroplasmic compartment with 5-HT, relative to many other amines and in conjunction with membrane and vesicle 5-HT uptake systems may contribute to the homogeneity of the neurotransmitter delivered to the synaptic cleft. Noradrenergic neurones contain both MAO-A and MAO-B. Furthermore, noradrenaline is a reasonably good substrate for both forms of the enzyme. However, since the affinity of the vesicular uptake mechanism for noradrenaline is much higher, uptake of noradrenaline into synaptic vesicles should be strongly favoured over degradation by MAO-A or MAO-B.

Xenobiotics that may be metabolised by the monoamine oxidases

Although MAO has been shown to oxidise many xenobiotics, the quantitative importance of its involvement in relation to other metabolic pathways is frequently unknown. High concentrations of xenobiotics that are MAO substrates may affect the metabolism of endogenous amines by competition. However, MAO activity appears to be present in excess in many tissues and a considerable level of inhibition (>80%) is required before substantial changes in brain monoamine concentrations, antidepressant effects and behavioural responses to administered amines, such as PEA, are observed.

XENOBIOTICS ALSO PRODUCED ENDOGENOUSLY: THE TRACE AMINES

These amines are present in relatively low amounts in the tissues and have sometimes been referred to as trace amines or micro-amines. They can be formed by the action of the enzyme aromatic-L-amino-acid decarboxylase (EC 4.1.1.28; sometimes also known as dopa decarboxylase) on the parent amino acids. Only in the case of 2-phenylethylamine, which appears to act by modulating the function of dopamine receptors, has there been much progress in defining physiological roles for these amines. They tend to be produced rather slowly and metabolised very rapidly in the tissues and the quantities arising from extraneous sources can be very much greater than the normal tissue levels.

Since bacterial aromatic-L-amino-acid decarboxylases are able to produce amines from the parent amino acids, it is not surprising that fermented and bacterially enriched foods contain a range of aromatic amines. Thus tyramine, tryptamine, histamine, 2-phenylethylamine, 5-HT and octopamine have all been found, in variable amounts, in foods and beverages such as wines, beers, cheese, salami, fermented cabbage, anchovy paste and soy sauce.

Tyramine is the most notorious of the xenobiotics metabolised by MAO and also the most well studied. This is because of the hypertensive reaction that occurs after ingestion of tyramine-rich foods or beverages by patients under therapy with MAO inhibitors (Blackwell and Marley 1969). Ingested amines such as tyramine are normally metabolised in the peripheral organs with the gastro-intestinal tract appearing to play the dominant role in this first-pass metabolism (Davis *et al*. 1984; Hasan

et al. 1988). As a result of this, very little ingested amine reaches the circulation. However, if MAO is inhibited, the ingested tyramine will enter the circulation from where it is actively taken up by peripheral adrenergic neurones, displacing stored noradrenaline and giving rise to a hypertensive response that can be fatal (Blackwell 1963). Since some cheeses are particularly rich in tyramine, this effect has become known as the 'cheese reaction'. Because of the widespread occurrence of tyramine in foods and beverages, the diets of patients being treated with such MAO inhibitors has had to be carefully restricted.

Lists of foods and beverages which may be high in tyramine have been complied (Sen 1969; Steward 1976; Da Prada *et al*. 1988). It should be noted, however, that the tyramine content of many foods and beverages can be extremely variable. Thus, Dostert (1984) has shown the tyramine content of beef liver to vary between 5 and 274 $mg.g^{-1}$ depending on the method and period of storage, and Da Prada *et al*. (1988) reported the tyramine content of some cheeses to depend on the state of ripeness. We have found values between 0.19 and 1.31 $mg.l^{-1}$ for different samples of beer from the same producer (Wheatley and Tipton 1987). Such variability may explain wide discrepancies in the reported tyramine content of certain products. For example, earlier reports that Chianti wine had extremely high levels of this amine have not been subsequently confirmed (Da Prada *et al*. 1988).

Hypertensive responses can be elicited by administration of tyramine alone to experimental animals or to human subjects but this effect is greatly potentiated following inhibition of monoamine oxidase. The pressor responses of subjects treated with monoamine oxidase inhibitors can be quite variable, perhaps as a result of individual variations in transport or metabolic efficiency (Bieck *et al*. 1988), and/or to the wide variability of the tyramine content of different products, discussed above. Because of this there is a risk that a patient treated with a monoamine oxidase inhibitor may experiment, by trying different proscribed foods and beverages and find no great adverse effects, only to consume a tyramine-rich sample at a later stage.

MAO-A activity predominates in rat and human (see Figure 4.4) intestine (Strolin Benedetti *et al*. 1983a; Hasan *et al*. 1988). Studies with dog intestinal loops (Davies *et al*. 1984) and with everted intestinal preparations (Hasan *et al*. 1988; Anderson *et al*. 1993) have shown that tissue to be capable of deaminating 70–80% of the tyramine during transport. Selective inhibitors of MAO-A, such as clorgyline, give a cheese reaction that is no less pronounced than that observed in the presence of a non-selective irreversible inhibitor of MAO-A and MAO-B. In contrast, but consistent with the relatively low MAO-B in the gastro-intestinal tract, selective inhibitors of MAO-B, such as *l*-deprenyl, do not potentiate the effects of dietary tyramine. Since MAO-A inhibitors are effective antidepressants whereas MAO-B inhibitors are not, the problem of producing dietary-safe antidepressant MAO inhibitors has been addressed by the development of reversible and competitive MAO-A inhibitors that can be administered in sufficient doses to give an antidepressant effect while being displaced by high concentrations of tyramine. Studies with several reversible MAO-A inhibitors, such as moclobemide and brofaromine, have indeed shown that their ability to cause a cheese reaction can be considerably lower than that observed with an irreversible inhibitor. The combination of a monoamine oxidase inhibitor with an inhibitor of presynaptic amine transport, such as amitriptyline, has been reported to reduce the

cheese effect (Pare 1986). Attempts have also been made to combine a monoamine oxidase inhibitor and an amine uptake inhibitor in the same molecule (Tipton *et al.* 1982). However, the pharmacokinetic behaviour of such hybrid molecules might be unsatisfactory if based on an irreversible MAO inhibitor and a reversible uptake inhibitor, such as one of the tricyclic antidepressants.

Long-term administration of MAO-inhibitory antidepressants can result in hypotension. This is believed to result from the gradual accumulation of tyramine and octopamine (see below), derived from it through the action of dopamine-β-hydroxylase (EC 1.14.17.1), which act as false transmitters.

2-Phenylethylamine (PEA) is present in many dietary sources and in particular at high concentrations in some, but not all, types of chocolate. It readily passes through membranes but is efficiently metabolised by both MAO and SSAO (semicarbazide-sensitive amine oxidase). The contribution of lung to the presystemic elimination of PEA was studied by Worland and Ilett (1983). The deamination of 10 μM PEA by whole tissue homogenates from rat suggested that the liver would have the major effect on its presystemic elimination, with a much smaller, approximately equal, contribution from the intestine and lungs. However, a comparison of the areas under the blood concentration–time curve after intra-arterial, intravenous, intraportal and intraduodenal administration of PEA (4 mmol.kg^{-1}) to the rat indicated that first-pass elimination of PEA was largely attributable to the intestine and lung, with a relatively small hepatic contribution.

Tryptamine is a substrate for both monoamine oxidases in human tissues, unlike 5-HT, which is a relatively specific MAO-A substrate. Since the parent amino acid may be administered in relatively high concentrations, as a nutritional supplement, an antidepressant and to assist sleeping, the possibility of increased bacterial and endogenous tryptamine formation cannot be ignored, but there are no quantitative data available on this.

Octopamine acts as a neurotransmitter in some invertebrates and specific octopamine receptors have been cloned from such sources. It has been used as an adrenergic drug but specific receptors have not yet been identified in mammalian systems. It appears to exert its effects by acting as a β_3-adrenoceptor agonist with a very much weaker effect at α_2-receptors. It is metabolised by both monoamine oxidases, but selective inhibition of rat brain MAO-A by clorgyline indicated it to be preferentially oxidised by MAO-A (Lyles 1978; Suzuki *et al.* 1979). As with phenylethylamine and phenylethanolamine, introduction of an hydroxyl group in the β-position in tyramine results in a decreased apparent affinity for MAO-A ($K_m \approx 115$ μM for tyramine and ~ 455 μM for octopamine, with rat brain).

Histamine is not a substrate for MAO. However it may be methylated to N^T-methylhistamine which is a substrate for MAO-B (Figure 4.2).

XENOBIOTIC PRIMARY AMINES (SEE FIGURES 4.2 AND 4.5)

Benzylamine, which is added to some mouthwashes and is also used in organic syntheses, is a highly selective substrate for monoamine oxidase-B and is also oxidised by SSAO. Its metabolite benzaldehyde is a narcotic at high concentrations and the further-oxidation product benzoic acid has antifungal properties. However, there is no

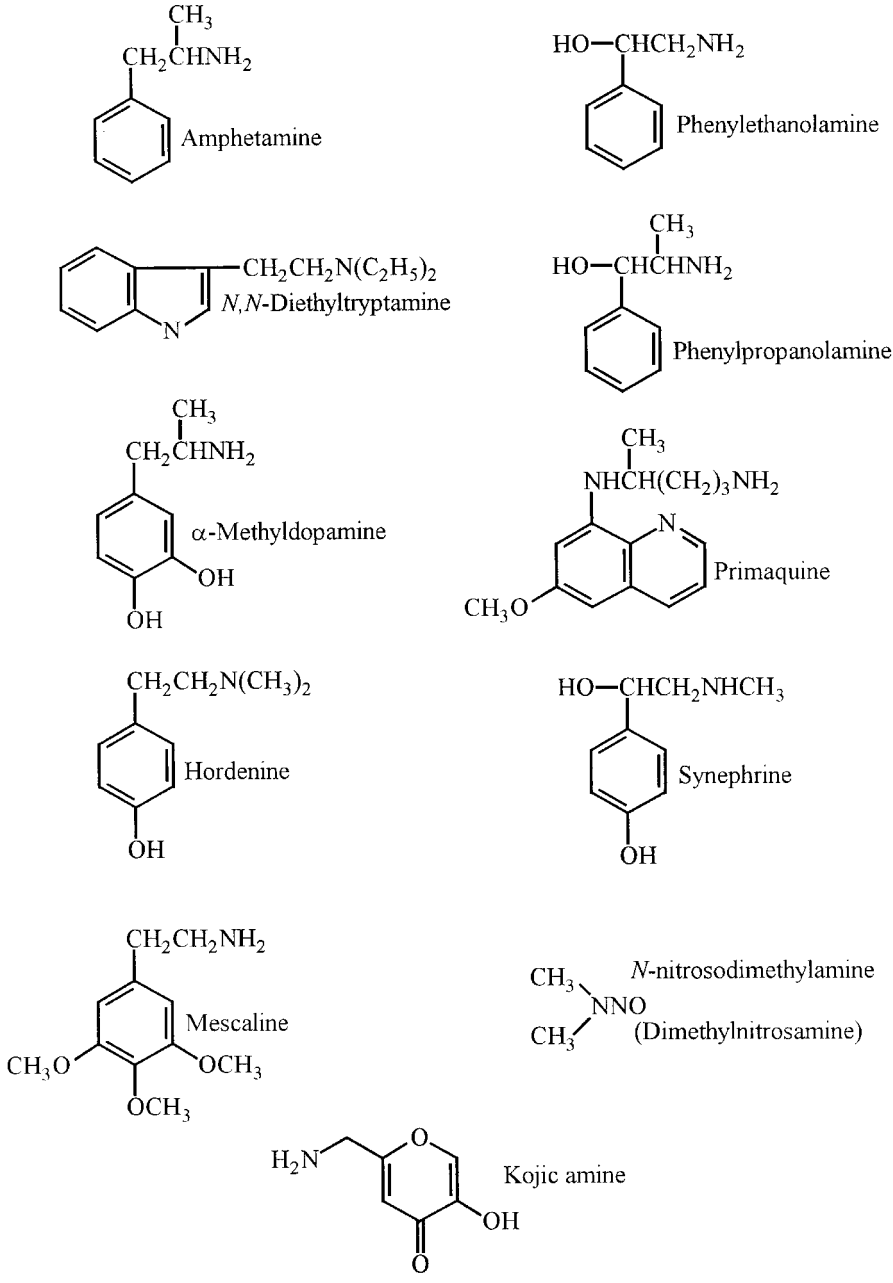


Figure 4.5 Some xenobiotics that have been reported to be amine oxidase substrates or inhibitors.

indication that sufficient quantities of this amine are consumed for these metabolite effects to be significant.

2-Phenylethanolamine is used in commercial processes and also as a topical vasoconstrictor. It is a selective substrate for MAO-B (Suzuki *et al*. 1979). The introduction of an hydroxyl group in the β-position results in it being more selective as a MAO-B substrate than 2-phenylethylamine, as a result of a decreased K_m value. In studies with the *R*- and *S*-enantiomers of 2-phenylethanolamine Williams (1977) reported that MAO-A would act only on the *R*-enantiomer whereas MAO-B would oxidise both enantiomers.

2-Phenylpropanolamine is present in many decongestants and cough medicines. Although tyramine is normally associated with the cheese reaction 2-phenylpropanolamine has also been shown to give rise to a hypertensive reaction in patients treated with monoamine oxidase inhibitors (Dollery *et al*. 1984).

Mescaline: MAO has been shown to play a significant role in the metabolism of mescaline in the mouse, since treatment with the non-selective MAO inhibitors iproniazid and tranylcypromine both resulted in a diminished formation of the acid metabolite, 3,4,5-trimethoxyphenylacetic acid and an increased formation of the alternative metabolite, *N*-acetylmescaline (Shah and Himwich 1971).

Primaquine: This antimalarial drug is metabolised to the corresponding carboxylic acid, carboxyprimaquine. Both MAO and the cytochrome P-450 monoxygenases can catalyse the oxidation of primaquine, but studies with rat liver homogenates indicated that inhibition of MAO with pargyline substantially reduced primaquine metabolism, whereas the cytochrome P-450 monooxygenase inhibitor SKF-525A (proadifen) had a lesser effect (Constantino *et al*. 1999). Thus it appears that, at least *in vitro*, MAO plays the dominant role in the oxidation of this drug.

Amphetamine and other α-methyl substituted amines are not substrates for the monoamine oxidases but are relatively effective reversible inhibitors that are selective towards MAO-A. Inhibitory potency towards MAO-A is stereoselective with the *S*-enantiomer of amphetamine being a more potent inhibitor of rat liver MAO-A than the *R*-enantiomer (Mantle *et al*. 1976), Earlier work, using selectively deuterium-substituted amines, showed that MAO abstracted the pro-*R*-hydrogen (H_{Re}) from the α-carbon of primary amine substrates (Belleau and Moran 1963) and, more recently, Yu *et al.* (1986) and Yu and Davis (1988) (see also Yu 1988) have shown both MAO-A and MAO-B to exhibit the same stereospecificity. The methyl group in *S*-amphetamine occupies the position of the non-abstracted hydon (H_{Si}) in the parent, non-methylated, substrate (2-phenylethylamine). However, the *R*-enantiomer of α-methylbenzylamine, in which the methyl group is in the position of the abstracted hydrogen (H_{Re}) in benzylamine, was reported to be a more potent inhibitor of both MAO-A and MAO-B from rat brain than the *S*-enantiomer (Arai *et al*. 1986). In the case of MAO-B there appears to be little difference in the potencies of the two enantiomers of either amphetamine (Dostert *et al*. 1989) or α-methylbenzylamine (Silverman 1984).

α-Methyldopa was introduced as an antihypertensive drug because of its ability to inhibit aromatic-L-amino-acid decarboxylase and hence decrease catecholamine formation. However, it is a substrate for that enzyme leading to the formation of α-methyldopamine which functions as a false transmitter and, *inter alia*, an inhibitor of MAO.

XENOBIOTICS METABOLISED BY WAY OF PRIMARY AMINES

Propranolol, a β-adrenoceptor blocker used as a cardiac depressant, has not been shown to be a substrate for MAO. However, it is N-dealkylated to the primary amine *N*-desisopropylpropranolol (DIP) by microsomal enzymes. The aldehyde product of the MAO-catalysed oxidation of DIP by rat liver mitochondria has been identified *in vitro*, as its *O*-methyloxime after reaction with methoxyamine hydrochloride, and its formation was shown to be inhibited by high, non-selective concentrations of the MAO inhibitor pargyline (Goldszer *et al*. 1981). Figure 4.6 summarises the metabolism of propranolol, by chemical decomposition of the aldehyde or by its further *in vitro* metabolism to the glycol and the acid derivative. Direct formation of the aldehyde from propranolol with loss of isopropylamine has also been shown to occur *in vitro*, but it appears to make a minor contribution to the overall process of oxidative deamination (Bakke *et al*. 1973). As a competitive MAO inhibitor propranolol was found to be selective towards MAO-A with an IC_{50} value towards 5-HT deamination in rat brain homogenates of 260 μM (Milmore and Taylor 1975). These results would be consistent with the compound being a selective substrate for MAO-A, but further studies would be necessary to establish whether that was the case. It appears that the *S*-enantiomer of propranolol is a better substrate for MAO than the *R*-enantiomer (Nelson and Bartels 1984). *S*-propranolol has the same absolute configuration as *R*-(−)-noradrenaline, which is a substrate predominantly for MAO-A in the rat (Strolin Benedetti *et al*. 1983b). It has not been directly demonstrated whether DIP is a selective substrate for either form of MAO.

A substantial contribution of MAO to propranolol metabolism has also been demonstrated *in vivo*. The carboxylic acid, 2-hydroxy-3-(1′-naphthyloxy)-propionic acid (Figure 4.6) is an important metabolite of propranolol in the human and in several other species, and the glycol metabolite has also been detected (Bargar *et al*. 1983; Walle *et al*. 1983). Side-chain oxidation and glucuronidation have been shown to be the dominant metabolic processes in the dog and human, whereas ring oxidation is dominant in the rat and hamster (Bargar *et al*. 1983; Walle *et al*. 1983). However, it is likely that the relative contributions of the various primary metabolic pathways (ring oxidation, side-chain oxidation and glucuronidation) to the overall propranolol metabolism will also depend on the dose.

Alprenolol and oxprenolol: The *in vivo* metabolism of alprenolol and oxprenolol (Figure 4.6) in the dog has been studied by Walle *et al*. (1981) and that of oxprenolol in the human by Dieterle *et al*. (1986). In the dog, the metabolic fates of alprenolol and oxprenolol were similar to that of propranolol. However, after oral administration of oxprenolol to humans, the direct *O*-glucuronidation pathway was found to predominate over the ring oxidation and side-chain oxidation pathways. The aldehyde, derived from the primary-amine metabolite, has also been detected following incubation of rat liver mitochondria with oxprenolol *in vitro* (Goldszer *et al*. 1981).

Pronethalol, timolol and other β-blockers: Tocco *et al*. (1980) showed the major metabolic pathway of timolol (Figure 4.6) in the dog to involve oxidation of the basic oxypropanolamine side chain to yield 2-hydroxy-3-([4-(4-morpholinyl)-1,2,5 thiadiazol-3-yloxy)propanoic acid, a pathway that is likely to involve MAO. However, in rat and human the two major metabolites of timolol are more highly oxidised,

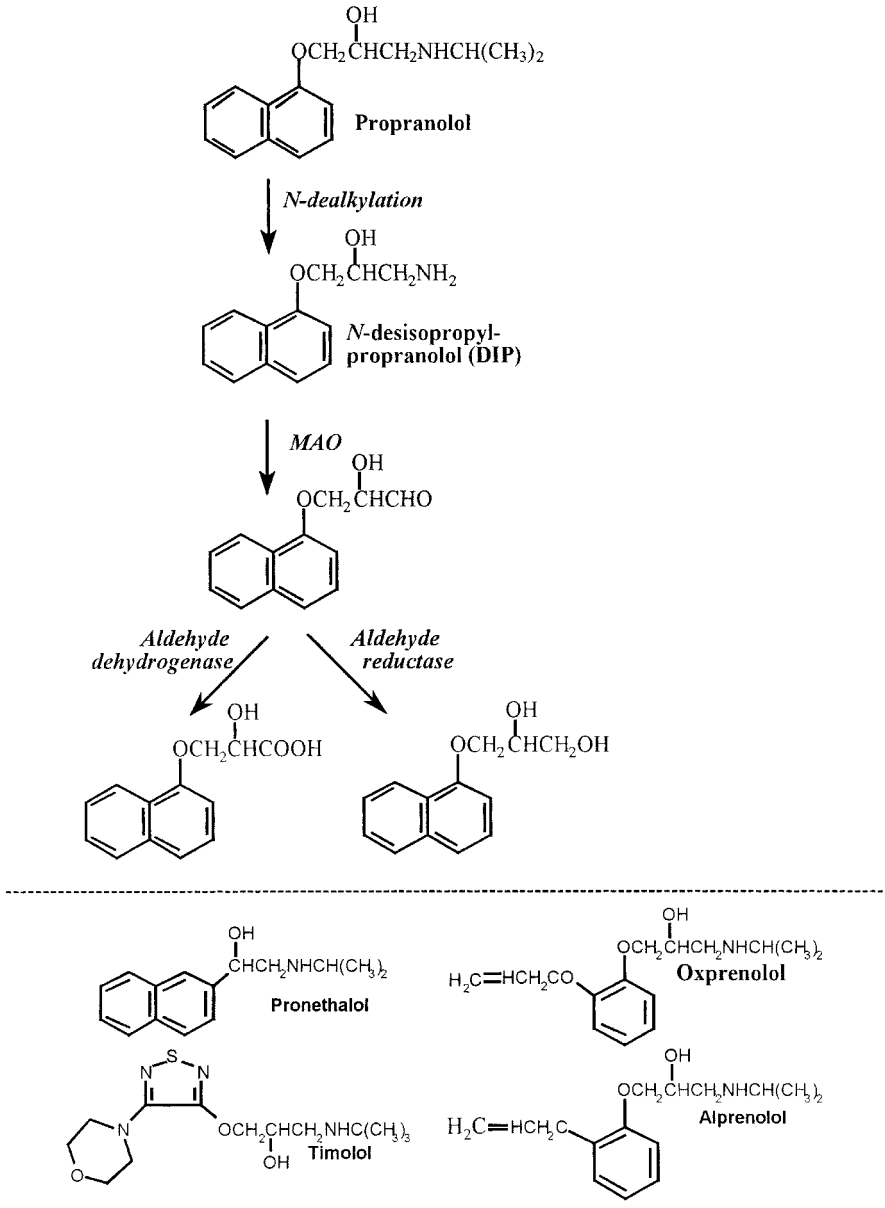


Figure 4.6 Involvement of MAO in the metabolism of propranolol. The structures of some other β-blockers, that are referred to in the text, are also shown.

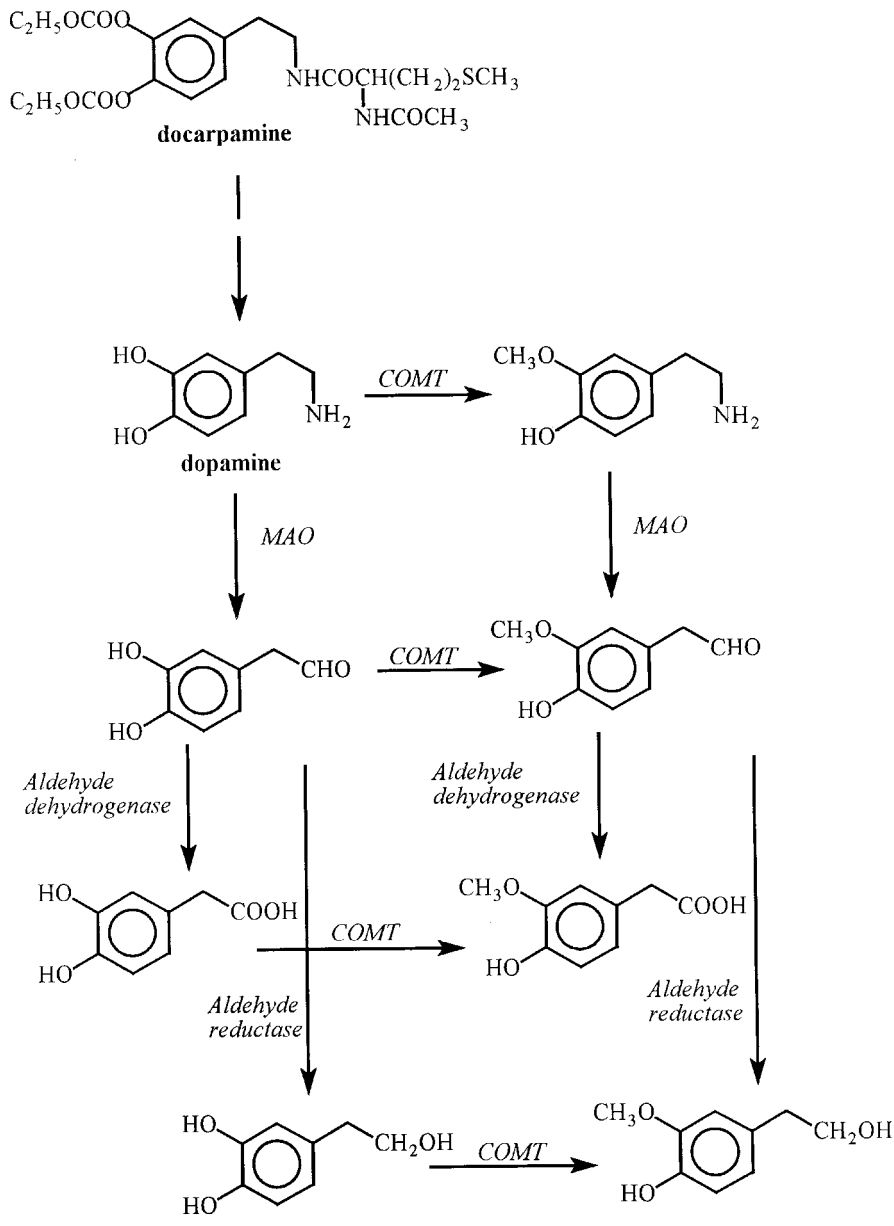


Figure 4.7 Formation of dopamine from docarpamine and its subsequent metabolism. The *O*-methylation reactions catalysed by catechol-*O*-methyltransferase (EC 2.1.1.6; COMT) have *S*-adenosylmethionine as the methyl donor, which is converted to *S*-adenosylhomocysteine.

morpholine ring-opened, species in which the oxypropanolamine side chain is unchanged (Figure 4.6). Bond and Howe (1967) also reported 2-naphthy-1-glycolic acid to be a major metabolite of pronethalol (Figure 4.6) *in vivo*. The probable involvement of MAO in the *in vivo* metabolism of K 5407 and several other β-blocking agents has also been demonstrated (Goldaniga *et al*. 1980).

Docarpamine (*N*-(*N*-acetyl-L-methionyl)-*O,O-bis* (ethoxycarbonyl) dopamine) is used as an orally active prodrug for the delivery of dopamine which can then be oxidatively deaminated by MAO (Figure 4.7), as indicated by the excretion of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in human urine (Yoshikawa *et al*. 1990). Studies in dog and rat have shown that the main site of catechol ester hydrolysis is the small intestine, whereas amide hydrolysis occurs in the liver (Yoshikawa *et al.,* 1995*).* The liver also catalyses extensive sulphoconjugation, although such sulphoconjugated dopamine is thought to be a possible precursor of active free dopamine in plasma (Tano *et al*. 1997).

SECONDARY AND TERTIARY *N*-METHYL AMINES

The MAO-catalysed oxidation of such compounds will yield the corresponding aldehyde plus methylamine. Methylamine is not a substrate for either MAO isoenzyme but, as discussed below, it is oxidatively deaminated by SSAO.

Synephrine is a vasopressor and adrenergic drug which has a somewhat lower K_m value for MAO ($\sim$ 250 μM) than its parent primary amine octopamine. Like octopamine it is oxidised by both forms of MAO, with a preference for MAO-B.

Epinine and ibopamine: dopamine has been used in the treatment of congestive heart failure (Goldberg 1989) because of its inotropic and vasodilating properties, but must be administered intravenously because it is poorly absorbed after oral administration (Goldberg 1974). This has led to the development of orally active derivatives of dopamine, such as ibopamine, which is hydrolysed by tissue esterases to epinine (Figure 4.8). Epinine (*N*-methyldopamine) is one of the few modifications of the neurotransmitter molecule dopamine (DA) that still retains agonist activity at the DA1 receptor. It is a substrate for both MAO-A and MAO-B with similar K_m and limiting velocity (V_{max}) values, although the apparent affinities for MAO-A and MAO-B ($K_m \approx 1000$ and 900 μM, respectively, in rat liver) are lower than those for dopamine ($K_m \approx 150$ and 290 μM, respectively). There is no evidence that ibopamine is oxidised by MAO (Strolin Benedetti *et al*. 1998).

Hordenine, the *N,N*-dimethyl derivative of tyramine, has a rather lower apparent affinity for rat liver MAO ($K_m \approx 1449$ μM) than synephrine and the use of selective MAO inhibitors has suggested it to be a selective substrate for MAO-B (Barwell *et al*. 1989).

***N,N*-Dimethyltryptamine** is an hallucinogenic compound that can be isolated from the leaves of some plants, such as *Prstonia Amazonica*. It is a specific substrate for MAO-B whereas the monomethyl derivative is a preferentially oxidised by MAO-A and, as mentioned above, the demethylated product tryptamine is metabolised equally well by both isoenzymes.

Citalopram: This antidepressant drug, a selective serotonin reuptake inhibitor (SSRI), may be demethylated to its *N*-desmethyl- and *N,N*-didesmethyl- (primary amine)

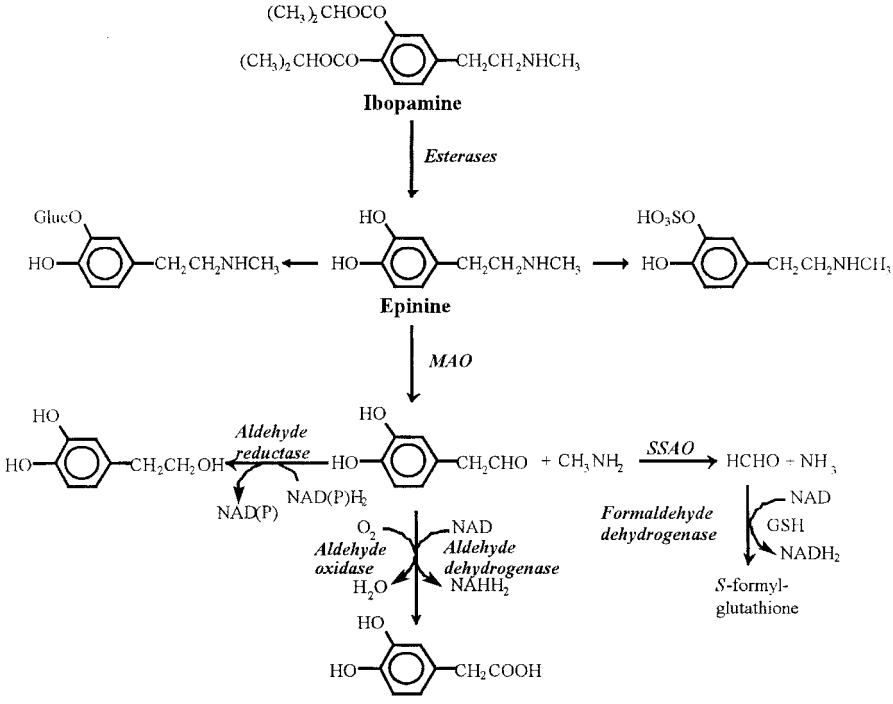


Figure 4.8 Formation of epinine from ibopamine and its subsequent metabolism. The *O*-methylated metabolites (see Figure 4.7) are omitted for clarity.

derivatives by members of the cytochrome P-450 family of monooxygenases. The role of MAO in its metabolism has been studied in detail by Rochat *et al.* (1998). Both the desmethyl-derivatives as well as citalopram itself are substrates for human liver MAO (Figure 4.9) and the predominant role of this enzyme in their metabolism *in vitro* was suggested by the substantial level of inhibition (>90%) by the non-selective MAO inhibitor phenelzine. The urinary excretion of the carboxylic acid metabolites of citalopram, *R*- and *S*-citalopram propionic acid, was detected after administration of racemic citalopram orally to human subjects, although the extraction procedures used involved the hydrolysis of any glucuroconjugates formed before analysis. The formation of *R*-citalopram propionic acid from racemic citalopram in human liver microsomal preparations, which also contained mitochondria, was shown to be substantially inhibited (87%) by low concentrations of clorgyline but to be rather insensitive (10.9%) to inhibition by low concentrations of *l*-deprenyl, indicating that MAO-A plays the dominant role in the oxidation of, the *R*-component of this substrate. MAO-A was also dominant in the formation of *R*-citalopram propionic acid formation from racemic *N*-desmethylcitalopram (65% inhibition by clorgyline) with about 16% inhibition resulting from *l*-deprenyl. In contrast, it appears that MAO-B plays the major

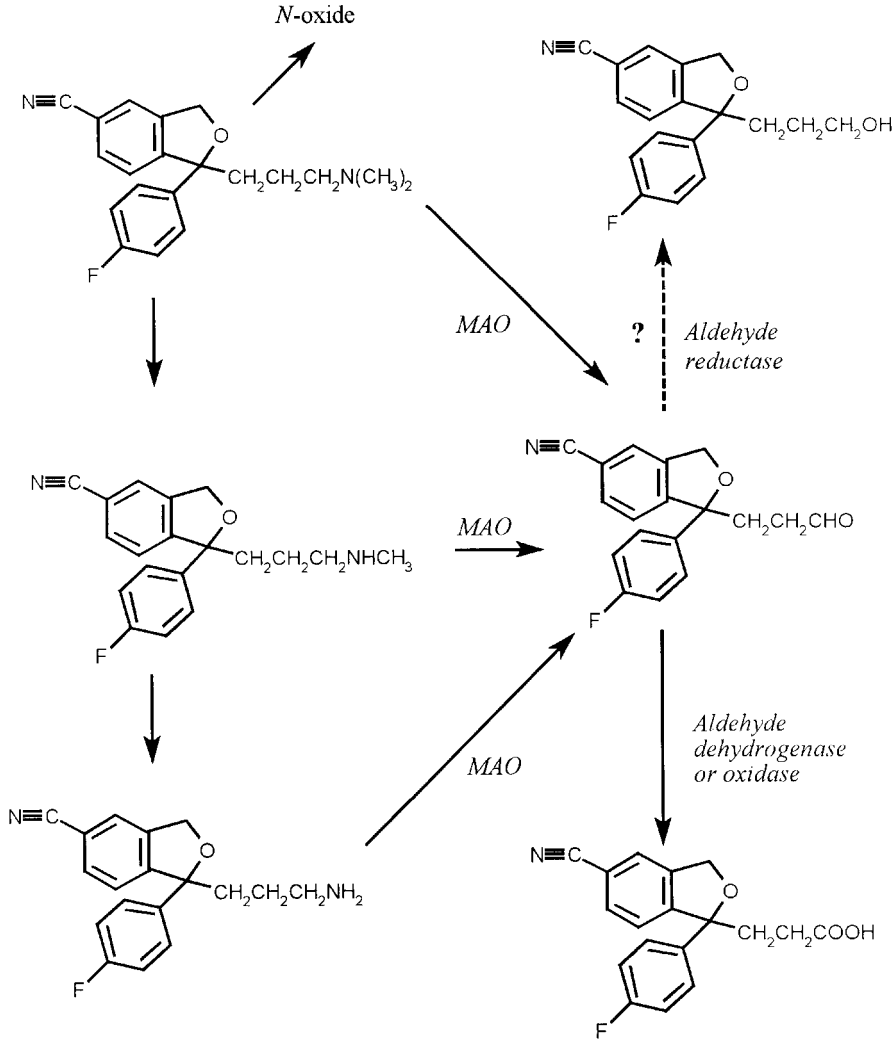


Figure 4.9 Metabolism of citalopram showing the involvement of monoamine oxidase. The primary alcohol product, which would be formed by aldehyde reductase or perhaps alcohol dehydrogenase, has not been detected.

part in metabolism of the primary amine metabolite, *N,N*-didesmethylcitalopram, with low concentrations of *l*-deprenyl and clorgyline resulting in 48.8% and 27.4% inhibition of *R*-citalopram propionic acid formation, respectively. MAO-B also appears to be more important in the metabolism of the *S*-component of racaemic *N,N*-didesmethylcitalopram with the formation *S*-citalopram propionic acid being

insensitive to low concentrations of clorgyline but approximately 93% inhibited by low concentrations of *l*-deprenyl. The corresponding figures for the involvements MAO-A and MAO-B in the formation of *S*-citalopram propionic acid from racemic *N*-desmethylcitalopram were 19.4% and 67.4% respectively, and 55% and 35.5% respectively from racemic citalopram.

A relatively minor role of the cytochrome P-450 monooxygenases in the formation of *S*-citalopram propionic acid from of *S*-citalopram was indicated by the observation that this process was inhibited by about 20% by the non-selective cytochrome P-450 monooxygenase inhibitor proadifen. This compound also inhibited to some extent the formation of *S*-citalopram propionic acid from the *S*-enantiomers of *N*-desmethylcitalopram and *N,N*-didesmethycitalopram, by about 25% and 34% respectively. However, these results are not easy to interpret, since proadifen also has a weaker inhibitory effect towards aldehyde oxidase (Robertson and Bland 1993; Watanabe *et al*. 1995). In contrast to these results with the *S*-enantiomers of citalopram and its desmethyl metabolites, the metabolism of the corresponding *R*-enantiomers to *R*-citalopram propionic acid was increased, up to 2.5 times, by proadifen, suggesting additional possible complexities in the metabolism of this enantiomer.

Triptans: The triptan family of compounds includes 5-HT receptor agonists that have been found to be of value in the relief of migraine. They are believed to act through the stimulation of presynapitc 5-HT_{1D} and related receptors to inhibit the release of calcitonin gene-related peptide and other peptides. In the case of sumatriptan (Figure 4.10) the major excreted metabolite in the human is the corresponding indoleacetic acid derivative and *in vitro* studies with human liver indicated that [^{14}C]-labelled sumatriptan was metabolised by MAO-A; there was no evidence of cytochrome P-450 monooxygenase involvement in its metabolism (Dixon *et al*. 1994). There are, however, species differences in the metabolism of sumatriptan. In humans, the indoleace-

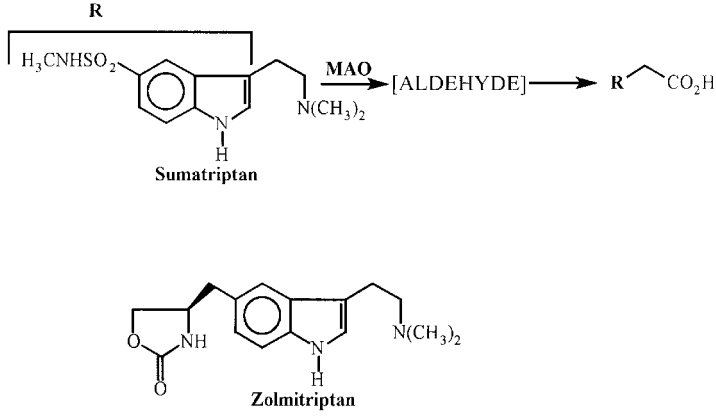


Figure 4.10 The metabolism of sumatriptan (Imitrex) by monoamine oxidase. The structure of zolmitriptan (zomig) is also shown for comparison.

tic acid metabolite is excreted partly as a glucuronide, whereas in animals such conjugation could not be detected. Furthermore, demethylation of the sulphonamide side chain of the drug occurred in rodent and lagomorph species but not in the human (Dixon *et al*. 1993).

There is also extensive first-pass metabolism of rizatriptan in humans. The major urinary metabolite was detected as being triazolomethyl-indole-3-acetic acid, although small amounts of urinary rizatriptan-*N*(10)-oxide, 6-hydroxy-rizatriptan and 6-hydroxy-rizatriptan sulphate could also be detected after high dosage (Vyas *et al*. 2000). Studies with the MAO-A selective inhibitor moclobemide indicated MAO-A to be the major metabolising enzyme in humans (Van Haarst *et al*. 1999). Three major metabolites of zolmitriptan, *N*-desmethyl-zolmitriptan, zolmitriptan *N*-oxide and the indoleacetic acid derivative, have been found *in vivo*. Studies with isolated human hepatocytes and liver microsomes have shown the conversion of zolmitriptan to *N*-desmethyl-zolmitriptan to be catalysed by CYP1A2 and not by MAO, whereas MAO-A is responsible for further metabolism of *N*-desmethyl-zolmitriptan (Wild *et al*. 1999).

Dimethylnitrosamine (*N*-nitrosodimethylamine) has been reported to act as a substrate for MAO (Lake *et al*. 1982), but in view of the difference between this substrate and any other known MAO substrate, this requires further investigation.

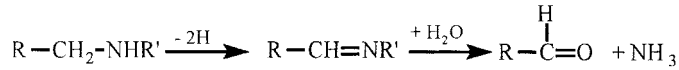
MAO inhibitors as substrates and MAO substrates as inhibitors

Many specific irreversible inhibitors are intrinsically unreactive compounds that are converted into reactive intermediates by the action of the enzyme itself. The inhibitor first forms a non-covalent complex with the active site of the enzyme and subsequent reaction within that complex leads to the generation of a reactive species that then reacts with the enzyme to form the irreversibly inhibited species. Inhibitors of this type are known as mechanism-based, enzyme-activated, k_{cat}, or suicide inhibitors. They can show a high degree of specificity towards a target enzyme because the generation of the effective inhibitory species from an essentially unreactive compound involves part of the catalytic function of the enzyme itself. Furthermore, the lack of intrinsic reactivity minimises the possibility of unwanted reactions with other tissue components.

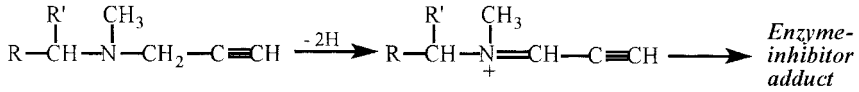
Figure 4.11 compares the irreversible inhibition of MAO by the acetylenic inhibitors with the oxidation of a normal amine substrate. In the latter case, the reduction of the enzyme-bound FAD results in the formation of an imine which is rapidly hydrolysed to ammonia plus the corresponding aldehyde. A similar oxidation of the acetylenic amine results in the formation of a doubly unsaturated derivative which acts as a Michael acceptor in reacting with an electron-rich group on the enzyme, in this case position 5 of the isoalloxazine ring of the covalently bound FAD, to form a flavocyanine. Kinetically the inhibition pathway can be represented by the mechanism (see Fowler *et al*. 1982)

$$\mathrm{E} + \mathrm{I} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{E.I} \xrightarrow{k_{+2}} \mathrm{E.I^*} \xrightarrow{k_{+3}} \mathrm{E\text{-}I} \qquad (4.1)$$

(a)



(b)



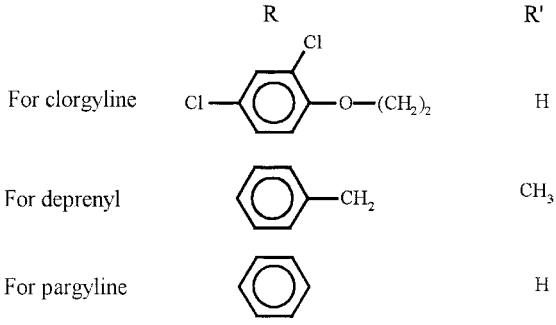
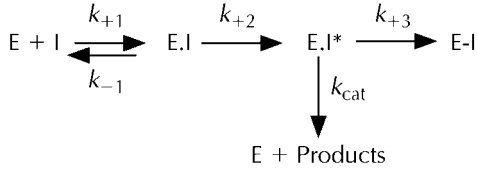


Figure 4.11 The oxidation of (a) substrates and (b) acetylenic (mechanism-based) irreversible inhibitors by MAO.

in which the inhibitor first forms a non-covalent complex (E.I) with MAO, and that complex is then transformed, by the action of the enzyme, into an activated species (E.I*) which then reacts with a group on the enzyme to form the irreversibly inhibited species (E-I).

Because mechanism-based inhibitors behave like substrates in binding to the active site of the enzyme and being converted to the reactive species through a process resembling the normal catalytic process of the enzyme, it is not surprising that it is possible for a proportion of the reactive species (EI*) to break down to form product. Compounds that behave in this way have sometimes been referred to as 'suicide-substrates'. In such cases, the formation of product and the mechanism-based inhibition of the enzyme will be competing reactions according to the following extension of the system shown above (Tipton 1989):



(4.2)

The relative effectiveness of a compound to act as a substrate or inhibitor can be defined as the *partition ratio* (*r*), which is the number of mol of product that is produced by 1 mol of enzyme before it is completely inhibited. Thus if a known amount of enzyme, [E], is incubated with excess of the substrate/inhibitor the activity of the enzyme will steadily decrease with time and the amount of product formed when all the enzyme has been inhibited, $[P_\infty]$, can be used to determine the partition ratio, according to the relationship:

$$\boldsymbol{r} = \frac{[P_\infty]}{[E]} = \frac{k_{cat}}{k_{+3}} \tag{4.3}$$

The number of mol of inhibitor necessary to inactivate 1 mol of enzyme will thus be given by $(1 + r)$ and so the initial concentration of inhibitor must be greater than $(1 + r)[E]$ for complete inhibition of the enzyme. If the inhibitor concentration is less than this, the amount of product formed will be given by $r.[E_i]$, where $[E_i]$ represents the concentration of inhibited enzyme.

Clearly compounds that act like this can either be regarded as substrates that also act as inhibitors, if the value of *r* is large, or as inhibitors that also act as substrates, if the value of *r* is small. However, the distinction is essentially arbitrary and there is no agreed criterion as to what constitutes a low, inhibitor, value of *r* and what constitutes a high, substrate, one. Thus the distinction made below is essentially arbitrary.

SUBSTRATES THAT ARE ALSO INHIBITORS

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

MPTP is a selective neurotoxin that causes a Parkinsonism-like syndrome in humans and some other species (for review see Tipton and Singer 1993) as a result of degeneration of the nigrostriatal pathway. MPTP is oxidised to the 1-methyl-4-phenylpyridinium ion (MPP^+), which is the active neurotoxin. In the brain the oxidation of MPTP takes place outside the dopaminergic nerve terminals since there appears to be little or no MAO-B within these structures (Westlund *et al*. 1985; O'Carroll *et al*. 1987*).* The active transport of MPP^+ into the dopaminergic nerve endings then results in it being concentrated there.

MPP^+ is an inhibitor of mitochondrial energy metabolism, at the level of NADH oxidase (Complex 1). Inhibition of mitochondrial function is not specific to brain mitochondria, but the specific uptake by dopaminergic nerve terminals maintains a sufficiently high concentration for sustained toxicity to result in cell death.

MPTP is oxidised to MPP^+ in a two-step process in which the dihydropyridine (1-methyl-4-phenyl-1,2-dihydropyridinium; $MPDP^+$) is formed as an intermediate (Figure 4.12). The oxidation of MPTP to $MPDP^+$ is similar to the initial oxidation step, imine formation, in the oxidation of primary amines by MAO. MAO can also catalyse the conversion of $MPDP^+$ to MPP^+ although this reaction can also occur non-enzymically (Singer *et al.*, 1985, 1986). However, MPTP also behaves as a time-dependent irreversible inhibitor as well as a substrate for MAO-B, according to the reaction shown in equation (4.2), which will limit the production of MPP^+ (Kreuger *et al*. 1990; Tipton *et al.,* 1986). It also appears that this enzyme may show

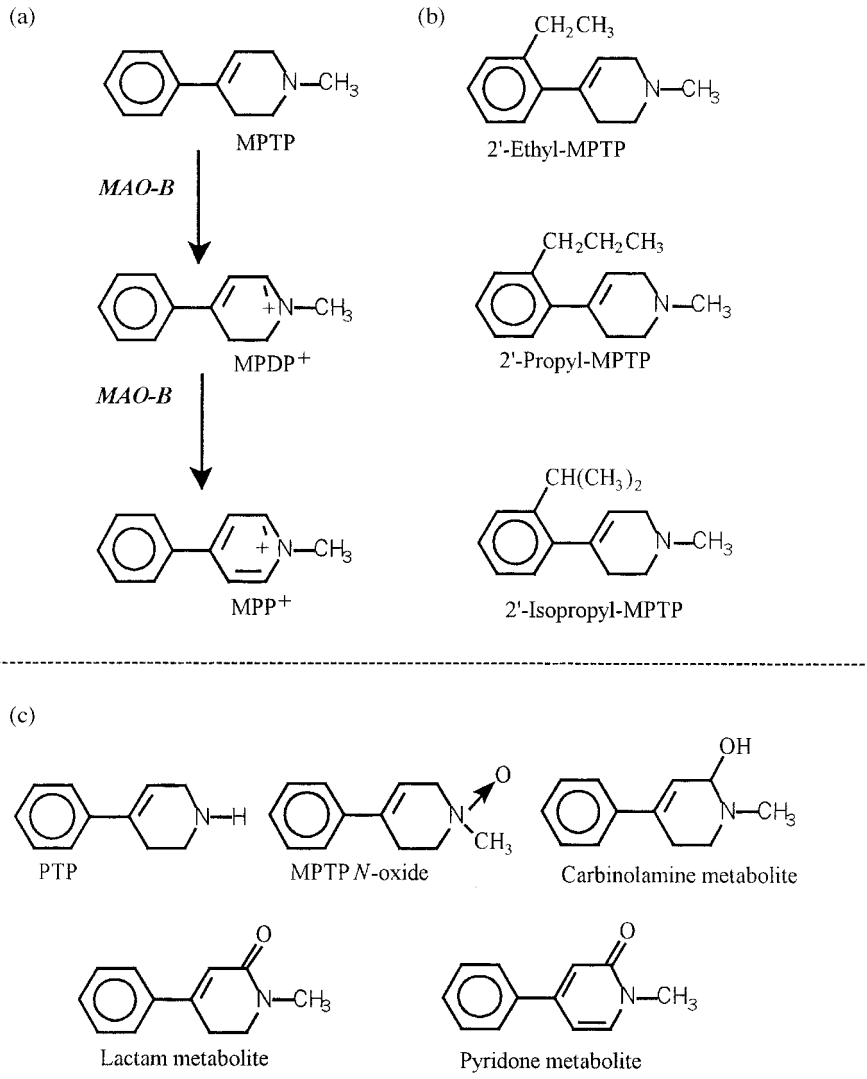


Figure 4.12 (a) The oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by MAO. (b) The structures of some analogues that are also substrates. (c) Some other metabolites that have been shown to be formed in liver. PTP = (4-phenyl-1,2,3,6-tetrahydropyridine; desmethyl-MPTP), which is also a substrate for MAO, and the carbinolamime metabolite are believed to be formed through the action of cytochrome P-450 monooxygenases on MPTP, whereas a flavin-dependent monooxygenase converts this compound to the *N*-oxide. The lactam and pyridone metabolites arise from the action of aldehyde oxidase on $MPDP^+$ and MPP^+, respectively.

large species-dependent differences in partition ratios with MPTP (Tipton *et al*. 1993; Sullivan and Tipton 1992) which may be one of the contributory factors to the wide species differences in sensitivities towards MPTP toxicity that have been reported (Tipton and Singer 1993).

Inhibitors of MAO-B, such as *l*-deprenyl, protect against the neurotoxicity of MPTP. The discovery that MAO-B was involved in the conversion of MPTP to the active toxin resulted in speculations that an endogenous toxin that was similarly formed through the action of this enzyme was involved in the aetiology of idiopathic Parkinson's disease. However, there is no *a priori* reason to believe that MAO-B is involved in the development of this disease. Studies on the interactions of MPTP analogues with MAO have shown that substitution of alkyl groups at the 2′ position of the benzene ring (Figure 4.12) increases the efficiency of oxidation by MAO-A, and lengthening the alkyl group in this position beyond CH_3 decreases the reactivity with MAO-B (Youngster *et al*. 1989). Thus the substitution of an ethyl or an isopropyl group in the 2′ position effectively converts a substrate for MAO-B into one that is preferentially oxidised by MAO-A. As might be anticipated from these results, the neurotoxicity of MPTP is prevented by pretreatment with deprenyl but not with clorgyline, that of 2′-methyl-MPTP requires both clorgyline and deprenyl to afford protection and clorgyline alone protects against the neurotoxicity of 2′-ethyl-MPTP (Heikkila *et al*. 1988). Furthermore, although some MAO-B inhibitors, such as *l*-deprenyl, have been shown to protect nerves against, or rescue them from, a number of potentially toxic insults, this occurs at concentrations below those necessary to inhibit the enzyme (for reviews see Tipton 1994; Olanow *et al*. 1998).

Although the conversion of MPTP to MPP^+ appears to be the dominant metabolic process in brain, there are alternative catabolic pathways in liver. Cashman and Ziegler (1986) showed that microsomal oxidation reactions competed with mitochondrial oxidation in rat liver. The microsomal cytochrome P-450 monooxygenases converted MPTP to nor-MPTP (PTP, desmethyl-MPTP), a compound that is not neurotoxic (Sullivan and Tipton 1990, 1992) whereas the primary product of the microsomal flavin-containing monooxygenase activity was MPTP-*N*-oxide. The kinetic parameters for the *N*-oxide formation from MPTP by rat liver microsomes were reported to be: $K_m = 45$ μM and $V_{max} = 4.8$ nmol.min^{-1}.mg of protein^{-1}. For comparison, K_m values for the oxidation of MPTP by monoamine oxidase B from rat and human liver are within the range 65–150 μM and the maximum velocities are similar to those for *N*-oxide formation (Tipton *et al*. 1986; Gessner *et al*. 1986). Cashman and Ziegler (1986) also examined the metabolism of MPTP in whole homogenates of human liver biopsy samples that were supplemented with NADPH. They found MPTP to be oxidised to approximately equal extent to MPTP-*N*-oxide and MPP^+, with nor-MPTP being formed to a lesser extent. These metabolites of MPTP formed *in vitro* by liver tissues are shown in Figure 4.12. The existence of alternative metabolic pathways for MPTP in liver will presumably also contribute to the insensitivity of peripheral tissues to permanent damage from MPP^+. In spite of the observation that the liver microsomal conversion of MPTP to nor-MPTP is some 20 to 25 times slower than *N*-oxide formation (Cashman and Ziegler 1986), the involvement of the cytochrome P-450 system in the metabolic process *in vivo* is illustrated by the significant decrease in the acute neurotoxicity of MPTP that occurs after induction of this system by

pretreatment of rats with phenobarbital (100 mg/kg, intraperitoneally for three days; Strolin Benedetti, unpublished results).

Monoamine oxidase has also been reported to catalyse the oxidation of *N*-methylated 1,2,3,4-tetrahydroisoquinolines and of their 6,7-dihydroxy derivatives to the corresponding quinolinium ions, which have been suggested to be endogenous Parkinsonism-inducing neurotoxins (Naoi *et al*. 1994), although one compound in this series was found to be oxidised by a semicarbazide-sensitive amine oxidase and not by MAO (Naoi *et al*. 1995).

Milacemide

The anticonvulsant drug milacemide (2-*n*-pentylaminoacetamide) (van Dorsser *et al*. 1983) has been shown to be a good substrate for monoamine oxidase-B but to be oxidised only poorly by MAO-A. Furthermore, acute administration of milacemide to rats was found to result in the urinary elimination of glycinamide, which was partly prevented by pretreatment *l*-deprenyl but not by clorgyline (Janssens de Varebeke *et al*. 1988). Oral administration of milacemide (100 $mg.kg^{-1}$) resulted in increased concentrations of glycine in rat forebrain, cerebellum and medulla (Christophe *et al*. 1983). A significant increase in glycine levels in rat cortex, cerebellum and hippocampus, but not in striatum and substantia nigra, was also reported after intraperitoneal administration of the same dose of milacemide (Chapman and Hart 1988). Thus, milacemide acts as a precursor of glycine in the brain and it has been suggested that this may account for its anticonvulsant actions (Christophe *et al*. 1983). The MAO-B catalysed conversion of milacemide to glycinamide, which subsequently breaks down to glycine, is shown in Figure 4.13. Milacemide also behaves as a time-dependent inhibitor of the enzyme, according to the mechanism shown in equation 4.2 (O'Brien *et al*. 1994a,b). There are, however, pronounced species differences in the partition ratios and kinetic parameters of MAO-B with milacemide and some of its analogues (Sullivan *et al*. 1990; O'Brien *et al*. 1995).

As discussed above, α-methyl-substituted amines are not effective substrates for MAO and the analogue α-methyl-milacemide (2-[(1-methyl)pentyl]aminoacetamide—see Figure 4.13) was found to be a competitive inhibitor with little selectivity towards either form of MAO (O'Brien *et al*. 1991). Since this compound was found to be an effective anticonvulsant in mice but, in contrast to milacemide itself, did not elevate urinary glycine levels, it was concluded that the MAO-catalysed oxidation of milacemide to form glycinamide was not a major factor in its anticonvulsant action but rather served to terminate that activity. This conclusion was supported by studies on the effects of a number of milacemide analogues in which the aminoacetamide portion was retained but the pentyl moiety was replaced with substituted-aromatic residues. Comparison of the abilities of these compounds to act as substrates and inhibitors of MAO revealed no simple correlations with their anticonvulsant activities, as measured by their ability to prevent bicuculline-induced convulsions and death in the mouse (O'Brien *et al*. 1994b). Yu and Davis (1990, 1991a,b) have investigated the potential of the milacemide analogues 2-propyl-1-aminopentane and 2-[(2-propyl)pentylamino]acetamide to deliver the anticonvulsant valproate to the brain. Both compounds were shown to be substrates for MAO-B, as shown in Figure 4.13. However,

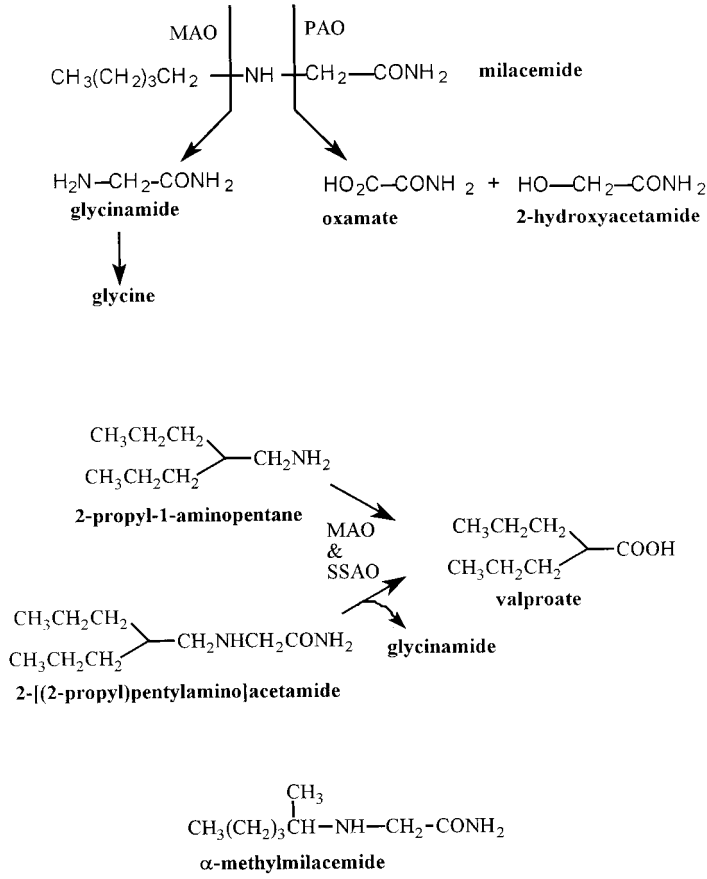


Figure 4.13 Patterns of oxidative cleavage of milacemide and its analogues 2-propyl-1-aminopentane and 2-[(2-propyl)pentylamino]acetamide by amine oxdases. Note: it has been reported that MAO-B from beef liver can form the cleavage products shown for PAO to a very minor extent (Silverman *et al.* 1993). The structure of α-methylmilacemide, which is not a substrate for MAO, is also shown.

although the appearance of valproate in brain could be detected following intraperitoneal administration of either compound, no anticonvulsant activity was manifested, indeed they were found to potentiate the convulsant effects of mercaptopropionic acid.

INHIBITORS THAT ARE ALSO SUBSTRATES

With clorgyline, (−)-deprenyl and pargyline there is no evidence for any product formation, irreversible reaction with the enzyme being apparently stoichiometric (Fowler *et al.* 1982).

Phenelzine (2-phenylethylhydrazine)

This is an irreversible inhibitor of MAO that has been widely used as an antidepressant. However, the conversion of ^{14}C-labelled phenelzine to phenylacetic acid by the rat, both *in vivo* and *in vitro*, in a process that was prevented by the MAO-inhibitors tranylcypromine and pargyline was shown many years ago by Clineschmidt and Horita (1969). Although a mechanism involving the oxidation of the hydrazine group to the corresponding azine derivative was subsequently proposed to explain its action as a mechanism-based inhibitor (Patek and Hellerman 1974), this offered no explanation of the apparent involvement of MAO in the conversion of phenelzine to phenylacetic acid. Studies of the effects of substitution of the hydrogens at carbon-1 of phenelzine by deuterium had shown that the *in vivo* effects of this inhibitor were significantly potentiated (Dyck *et al.* 1983). This might be the result of the isotope hindering the removal of a hydrogen from the carbon-1 position. An oxidation at this position would be expected to result in the formation of the corresponding hydrazone (Tipton and Spires 1971) as an alternative to formation of the inhibitory azine. Subsequent hydrolysis of this compound, perhaps catalysed by the enzyme itself, would result in the formation of the corresponding aldehyde in a manner analogous to that occurring during the oxidation of primary amines by MAO. Studies on the formation of 2-phenylacetaldehyde during the incubation of MAO with phenelzine or 1,1-dideutero-phenelzine, by high-performance liquid chromatography, showed that the deuterium substitution resulted in a decrease in the formation of this product in a way that parallelled the increased potency as a time-dependent inhibitor of the enzyme (Yu and Tipton 1990). These results show that the inhibitory reaction and that leading to product formation are competing reactions according to the scheme shown in equation (4.2), as shown in Figure 4.14. In this mechanism the isotope effect caused by the substitution of deuterium for hydrogen at the side-chain carbon-1 position will result in a decrease in the C–N dehydrogenation without affecting the N–N dehydrogenation, thus favouring the pathway leading to irreversible inhibition over that

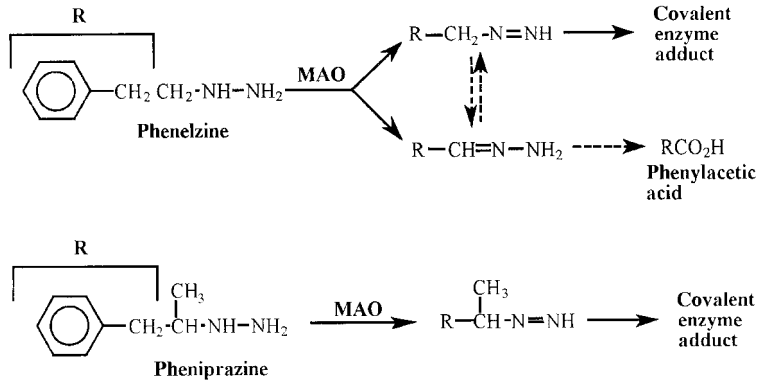


Figure 4.14 Phenelzine ([2phenethyl]hydrazine), as a substrate and inhibitor of MAO and its α-methyl derivative, pheniprazine ([1-methyl-2phenethylhydrazine), as an inhibitor.

leading to product formation. Such deuterium isotope effects may constitute a method for the design of more effective monoamine oxidase inhibitory antidepressants.

A number of different hydrazine derivatives have been used as MAO inhibitory antidepressants (Tipton 1990) but it is not known whether many of these were also substrates for the enzyme. As with other α-methyl substituted amines, the α-methyl derivative of phenelzine, pheniprazine (1-methyl-2-phenylethylhydrazine, see Figure 4.14), is not oxidised by MAO to any detectable extent, although it is a mechanism-based inhibitor with a (relatively weak) selectivity towards MAO-A (Ben Ramadan and Tipton 1999).

MD 780236, MD 240928 and MD 240931

The monoamine oxidase inhibitor 3-(-4((3-chlorophenyl)-methoxy)-phenyl)-5-methylamino)methyl-2-oxazolidinone methane sulphonate (MD 780236) follows the mechanism shown in equation (4.2), (Figure 4.15), and a partition ratio of about 530 has been determined with rat liver monoamine oxidase-B (Tipton *et al*. 1983). The formation of the aldehyde product as well as of the carboxylic acid and alcohol metabolites have been shown both *in vivo* and *in vitro* (Strolin Benedetti *et al*. 1983b; Tipton *et al*. 1984a). In contrast , MD 780236 acts as a substrate for MAO-A *in vitro* in the rat (Tipton *et al*. 1983). Consistent with this, pretreatment of rats with the selective MAO-A inhibitor clorgyline enhances the irreversible inhibition of MAO-B by MD 780236 (Strolin Benedetti and Dow 1983).

MD 780236 is a racaemic compound and the configurations of its *R*- and *S*-enantiomers (MD 240928 and MD 240931, respectively) are shown in Figure 4.15. The interaction of the *S*-enantiomer (MD 240931) with MAO-B behaves according to the 'suicide-substrate' mechanism shown in equation (4.2) and it is this component that will be mainly responsible for the irreversible inhibition of MAO-B by MD 780236 observed *in vivo* (Dostert *et al*. 1983). In contrast, with the *R*-enantiomer (MD 240928), the breakdown of the activated enzyme-inhibitor complex to give products occurs very much more rapidly than the reaction to produce irreversible inhibition, so that it acts essentially as a substrate for MAO-B. It has also been shown that the alcoholic products formed by the metabolism of MD 780236 and its enantiomers are potent MAO-B-selective inhibitors (Dostert *et al*. 1983; Tipton *et al*. 1983, 1984b). Thus the reversible inhibition of MAO-B by MD 240928 that has been observed *in vivo* (Turkish *et al*. 1988; Dostert *et al*. 1983) represents it competing as a substrate for the enzyme plus reversible inhibition from any of the alcoholic metabolites that may accumulate.

A comparative study of the metabolic fate of radioactively-labelled MD 240928 showed that urinary elimination accounted for 32% 17% and 57% of the orally administered drug in rat, dog and human, respectively, and that most of the elimination occurred within 24 h. By this time, the unconjugated acid derivative accounted for most of the urinary radioactivity in the three species. The debenzylated derivative was also an important metabolite in rat urine. The alcohol derivative, both free and conjugated, was only as a minor component in the urine of the three species. The debenzylated derivative of the alcohol, accounted for 14–18% of the urinary radioactivity in the dog but was only a minor component in rat and human urine.

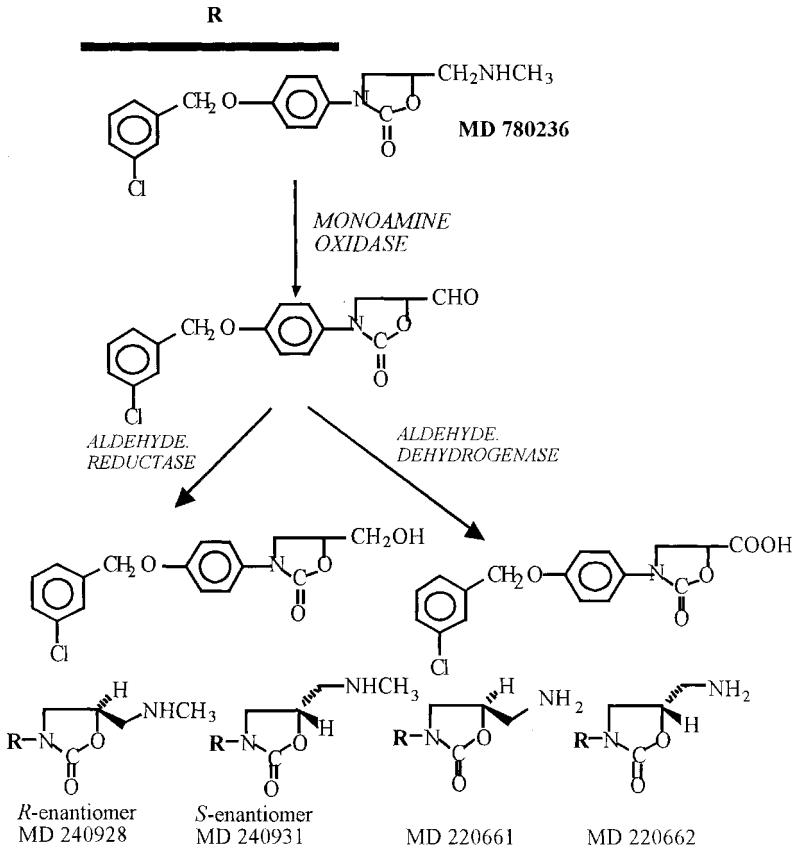


Figure 4.15 Metabolism of MD 780236 {3-[4-((chlorophenyl)-methoxy)-phenyl-5-[(methyl-amino)methyl]-2-oxazolidinone}. The configurations of the *R*- and *S*-enantiomers, MD 240928 (almoxatone) and MD 240931, are also shown.

Unchanged drug and its *N*-demethylated derivative were practically absent from the urine of the three species (see Figure 4.15). The acid derivative was the predominant form found in blood plasma, whereas in brain, which, after 2 to 4 hours, contained 0-2-0.3% of the radioactive dose administered, the alcohol derivative and the primary amine appeared to be the most important metabolites. Lower amounts of the acid derivative and the unchanged drug were, however, also found to be present in brain (Strolin Benedetti *et al.* 1984).

An additional complexity in the interpretation of the actions of MD 780236 and its enantiomers is that they are also rather weak, reversible inhibitors of SSAO (Dostert *et al.* 1984; Kinemuchi *et al.* 1986).

Moclobemide

This compound was developed as a reversible selective MAO-A inhibitor for use as an antidepressant with a minimal cheese effect (Haefely *et al*. 1992). It is a relatively weak MAO inhibitor *in vitro* but is considerably more potent *in vivo*. When preparations of MAO-A are incubated with moclobemide there is an initial low-affinity binding followed by a time-dependent increase in inhibition. This inhibition is, however, reversible by dialysis at 37 °C and, furthermore, if the enzyme-moclobemide mixture is incubated for extended times there is slow recovery of enzyme activity (see Haefely *et al*. 1992) Such behaviour might be explained by moclobemide acting as a very poor substrate for MAO-A according to the mechanism:

$$E + I \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E.I \xrightarrow{k_{+2}} E.I^* \xrightarrow{k_{+3}} E + \text{Products} \qquad (4.4)$$

where $k_{+3} \ll k_{+2}$ and the rate governed by k_{+2} is, itself, slow. However, there is extensive metabolism by other enzymes *in vivo* which yields additional MAO-inhibitory products (Cesura *et al*. 1990; Schoerlin and Da Prada, 1990).

RO 16-6491 is also a substrate for MAO-B. After incubation in the presence of the tritiated compound, the radioactivity bound to brain mitochondria and platelet membranes was extracted and analysed by HPLC (Cesura *et al*. 1987). Only a minor peak of radioactivity was eluted as RO 16-6491 (Figure 4.3). The major amount of radioactivity was attributed to the aldehyde derivative (RO 19-7731) whereas a small amount had the same retention time as the acid derivative (RO 11-1903).

The semicarbazide-sensitive amine oxidases

The situation concerning the semicarbazide-sensitive amine oxidases is confusing, as this term covers a rather diverse group of enzymes which also appear to differ significantly between tissues and species. Since they are often distinguished simply in terms of their sensitivity to inhibition by semicarbazide and insensitivity to the acetylenic monoamine oxidase inhibitors, it is, perhaps, not surprising that more than one enzyme is detected and it is not always clear from publications which enzyme is being studied. SSAOs can be found throughout the animal kingdom as well as in plants and micro-organisms. In vertebrates, members of the group include both plasma- and tissue-bound enzymes together with specifically named enzymes such as lysyl oxidase (Smith-Mungo and Kagan 1998), which differs from the other members of this group in having lysine tyrosylquinone rather than TOPAquinone as cofactor (Wang *et al*. 1997), and diamine oxidase (see Callingham *et al*. 1995; Houen 1999; Klinman and Mu 1994; Lewinsohn 1984; Lyles 1995, 1996 for reviews on different aspects of these enzymes).

The plasma- and tissue-bound SSAOs that are active towards primary monoamines have more restricted specificities than the monoamine oxidases; adrenaline, for example, is not a substrate. The best-known substrate for both the plasma- and tissue-bound amine oxidases is the non-physiological amine benzylamine. Indeed, plasma SSAO has sometimes been referred to as benzylamine oxidase. Its specificity for physiologically occurring amines overlaps with that of MAO, and 2-phenylethylamine

(PEA), benzylamine, tyramine and dopamine are all oxidatively deaminated by the plasma and tissue-bound SSAO in most species. Although 5-HT is not a substrate for SSAO from most sources, it is a good substrate for the enzyme from pig and human dental pulp (Nordqvist *et al*. 1982). Octopamine and phenylethanolamine have been reported not to be substrates (Elliott *et al*. 1989b) and, as with the monoamine oxidases, α-methyl substituted amines are not oxidised. It appears that there may be more than one SSAO enzyme in plasma from some species (Boomsma *et al*. 2000; Elliott *et al*. 1992), which further complicates attempts to define specificities.

The fact that blood from many species contains significant amounts of SSAO activity means that caution is necessary in interpreting results obtained with isolated-cell systems that are maintained in media containing foetal calf serum. It has been shown that the behaviour of some compounds that are SSAO substrates can be significantly altered by the enzyme that is present in the culture medium (Conklin *et al*. 1998; Inoue *et al*. 1990).

The tissue-bound enzyme is associated with the plasma membrane and it appears that a proportion of the active sites are exposed to the extracellular milieu (Holt and Callingham 1994). Thus, unlike the situation with MAO, substrates would not have to enter the cell for deamination by either plasma- or tissue-bound SSAO. The rapid disappearance of dopamine and, to a lesser extent, noradrenaline when added to blood plasma from several mammalian species has been attributed to SSAO (Boomsma *et al*. 1993).

Amines that are good substrates for SSAO but are not oxidised by MAO include aminoacetone and methylamine (see Lyles 1995, 1996 for reviews). Its activity, which is high in cardiovascular tissue, is elevated in diabetes, congestive heart failure and following severe burns (Lewinsohn 1977). Elevated levels of SSAO have also been found in the plasma of toxin-induced diabetic rats and sheep (Hayes and Clarke 1990; Elliott *et al*. 1991) and of non-insulin-dependent diabetes in humans (Meszaros *et al*. 1999). SSAO activity is also increased in brown adipose tissue of obese Zucker rats (Barrand and Callingham 1982). A regulatory link between the glucose transporter GLUT 4 and SSAO has recently been reported and this may have important implications for SSAO in non-insulin-dependent diabetes and other disorders involving glucose transport (Enrique-Tarancon *et al*. 1988, 2000). The enzyme is also apparently identical to vascular-adhesion protein 1 (VAP-1), an endothelial glycoprotein that supports adhesion of lymphocytes to hepatic endothelium (Smith *et al*. 1998). It has been postulated that the increased SSAO levels seen in some pathological conditions may represent the need for increased VAP-1 to mediate repair (Kurkijarvi *et al*. 2000; Bono *et al*. 1999).

One of the problems which has limited our understanding of SSAO has been the lack of potent, selective inhibitors of these enzymes. Excluding semicarbazide and related compounds, which inhibit many different enzymes, SSAO tends to share a number of its inhibitors with MAO, making discrimination between amino oxisases *in vivo* difficult and further emphasising the need for new and more specific inhibitors. The compound MDL 72145 ((E)-2-(3′, 4′-dimethoxyphenyl)-3-fluoroallylamine), which has been shown to be a potent irreversible inhibitor of SSAO in rat aorta (Palfreyman *et al*. 1994; Lyles and Fitzpatrick 1985) and brown adipose tissue (Elliott *et al*. 1989a), has been used in a number of studies on the metabolic role of SSAO.

However, it is also a potent inhibitor of MAO-B and affects MAO-A activity to a lesser extent (Zreika *et al*. 1984). The compound MDL 72274A ((*E*)-2-phenyl-3-chloroallylamine) appears to be highly selective towards SSAO, whereas MDL 72974A ((*E*)-2-(4-fluorophenethyl)-3-fluoroallylamine) is a potent inhibitor of both MAO and SSAO. However, these compounds have been little used for metabolic studies to date. Another possibility is the anticancer drug procarbazine (*N*-isopropyl-α-(2-methyl hydrazino)-*p*-toluamide hydrochloride), which together with its metabolite monomethylhydrazine appears to be highly selective for SSAO both *in vivo* and *in vitro* (Holt and Callingham 1995, Holt *et al*. 1992). However, it appears that there may be species and tissue differences in the sensitivities of amine oxidases to inhibition by hydrazine derivatives (Lizcano *et al*. 1996); again indicating the difficulty of extrapolating results obtained in one system to another.

COMPOUNDS THAT ARE SUBSTRATES FOR SSAO AS WELL AS MAO

Xenobiotic and endogenous amines

The involvement of SSAO in the metabolism of amines that are substrates for both enzymes has been less easy to establish because wide species differences in specificity and amount of enzyme present. For example, high activity is found in pig and sheep plasma, the levels are very much lower in human plasma and it is often difficulty to detect any at all in the rat. The levels of the tissue-bound enzyme also vary widely between species but do not parallel those of the plasma enzyme (Boomsma *et al*. 2000). Thus attempts to extrapolate from the situation in experimental animals to that in the human should be interpreted with great caution. Differences in substrate specificity between the SSAO enzymes from different mammalian sources appear to extend to stereospecificity. Alton *et al*. (1995; see also Palcic *et al*. 1995) reported the oxidation of benzylamine by plasma SSAO from bovine, horse, porcine, rabbit, and sheep plasma to involve abstraction of the pro-*S* hydrogen. In contrast, SSAO fom bovine plasma (Yu and Davis 1998) and from human aorta and plasma (Yu *et al*. 1994) has been repored to show no absolute stereospecificity in this respect.

The relatively high levels of SSAO in blood vessels suggest that it may be involved, perhaps in concert with MAO, in regulating the levels of circulating amines. For example, the pressor effects of tyramine in the perfused mesenteric arterial bed from the rat was found to be potentiated if both MAO and SSAO were inhibited. However, the response to the amine was unaffected by inhibition of either enzyme alone (Elliott *et al*. 1989c,d). In contrast, tryptamine-induced contraction of rat aorta was unaffected by SSAO inhibition but enhanced by combined inhibition of MAO and SSAO (Taneja and Lyles 1988). Furthermore, SSAO inhibitors alone can potentiate the contractile effects of sympathomimetic amines on the rat anococcygeus muscle preparation (Callingham *et al*. 1984). However, although SSAO is present in the intestine, it appears from studies with selective inhibitors that it does not play a significant role in limiting the effects of dietary tyramine, even in situations where MAO is inhibited (Hasan *et al*. 1988; Elliott *et al*. 1989d).

The lung from most species, including the rabbit, contains relatively high levels of SSAO activity and since, as discussed above, that organ can play a major role in the

metabolism of presystemic elimination of amines such as 2-phenylethylamine, that enzyme might be expected to be involved in the process. However, from studies that used pargyline and semicarbazide, Gewitz and Gillis (1981) concluded that SSAO did not play a significant role in the metabolism of this amine in the perfused rabbit lung.

XENOBIOTIC AMINES

Milacemide analogues

The formation of glycinamide from milacemide appears to be specific to monoamine oxidase since it has been shown not to be a substrate for the semicarbazide-sensitive amine oxidase (Strolin Benedetti *et al*. 1988). However, the milacemide analogues 2-propyl-1-aminopentane and 2-[(2-propyl)pentylamino]acetamide (Figure 4.13) were both found to be substrates for SSAO from rat aorta (Yu and Davis 1990, 1991a) forming 2-propyl-1-pentaldehyde which could be subsequently oxidised to valproate. The quantitative significance of this, relative to the activity of MAO-B towards these substrates, is unclear. The observation that the acetamide derivative is a substrate was unexpected in view of the commonly-held view that the activity of this enzyme is restricted to primary amines.

Mescaline

Over 40 years ago, Blaschko *et al*. (1959) showed mescaline to be a substrate for pig plasma SSAO. Indeed, mescaline is more efficiently deaminated than benzylamine in pig plasma (Buffoni and Della Corte 1972). Roth *et al*. (1977) reported that the mescaline-oxidising activity of rabbit lung homogenates was two to three times greater than that of either liver or kidney and that brain and plasma had comparatively little capacity to metabolise mescaline. They showed mescaline metabolism by the perfused rabbit lung to be sensitive to inhibition by semicarbazide but not by pargyline (1 mM). Since mescaline efflux from the perfused lung was slower than that of its 'metabolite', presumed to be the carboxylic acid, these results were interpreted to indicate that the intact lung efficiently removes perfused mescaline and may be important in the disposition of circulating mescaline *in vivo*. Unfortunately, the situation in the human is not clear, and mescaline does not appear to be a substrate for the human plasma enzyme. Jacob and Shulgin (1981) reported that a number of thiol analogues of mescaline and isomescaline (2,3,4-trimethoxyphenethylamine) were substrates for beef plasma SSAO but provided no information on the activities of MAO towards them.

Primaquine

The possible contribution of SSAO to the metabolism of this antimalarial drug, which has been shown to be a substrate for the pig plasma enzyme (Blaschko and Hawes 1959), remains uncertain. However, as discussed above, it appears that in rat liver, at least *in vitro*, the contribution of monoamine oxidase is dominant.

COMPOUNDS THAT ARE SUBSTRATES FOR SSAO BUT NOT FOR MAO

Methylamine

This is produced physiologically from a number of catabolic reactions, such as the breakdown of adrenaline, catalysed by monoamine oxidase, sarcosine and creatine. However, since it is also a common component of some foods and beverages (Lichtenberger *et al.* 1991; Lin *et al.* 1984) and is also an atmospheric pollutant and present in cigarette smoke (Yu 1998a), it can be regarded as a xenobiotic that is also produced endogenously. Methylamine is not a substrate for MAO (Yu 1989) but is oxidised by SSAO to form ammonia, hydrogen peroxide and formaldehyde (see Figure 4.16). It has been argued that the high levels of SSAO in lung are to protect against inhaled methylamine and other volatile amines (Lizcano *et al.* 1990, 1998). However, formaldehyde is an extremely reactive chemical that can produce irreversible adducts with proteins and single-strand DNA, among other harmful cross-linkage reactions (Yu 1998a,b). It is normally metabolised to formate by formaldehyde dehydrogenase in the presence of reduced glutathione. Interestingly, serum does not contain any formaldehyde dehydrogenase and any formaldehyde produced in the blood cannot be metabolised until it is transported into the erythrocytes. This may be significant in terms of formaldehyde-induced toxicity in blood vessels.

The consumption of relatively large amounts of creatine as a nutrition supplement, in attempts to enhance sports performance, would lead to increased methylamine and, hence, formaldehyde and H_2O_2 production from its catabolism. This might underlie some of the deleterious effects of long-term creatine consumption (Yu and Deng 2000).

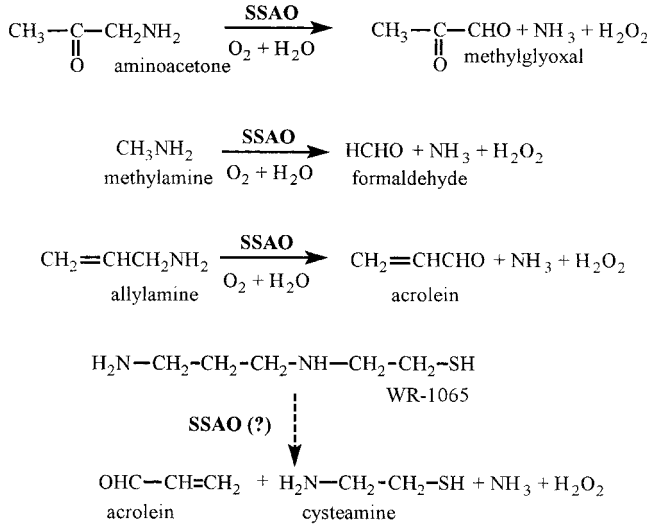


Figure 4.16 Oxidation of allylamine, aminoacetone and methylamine by SSAO. The oxidation of WR-1065 (2-(3-aminopropylamino)ethanethiol) is also shown.

Kojic amine

Kojic amine (2-(aminomethyl)-5-hydroxy-4H-pyran-4-one; see Figure 4.5) is a GABA (γ-aminobutyric acid) receptor agonist that has been reported to be a substrate for SSAO (Ferkany *et al*. 1981), but there are no data on the metabolic significance of this. Neither is it known whether MAO also oxidises this compound.

4-S-Cysteaminylphenol (4-S-CAP)

This compound was developed as an inhibitor of melanoma growth. Its cytotoxicity is largely due to the formation of dihydro-1,4-benzothiazine-6,7-dione (dihydro-1,4,benzothiazine-quinone), which is catalysed by tyrosinase and also occurs by autoxidation (Hasegawa *et al*. 1997). However, it appears that SSAO can also catalyse the conversion of this compound to the corresponding aldehyde, which may then be converted to the corresponding acid and alcohol metabolites (Inoue *et al*. 1990), as shown in Figure 4.17. Aldehyde formation in this way appeared to enhance the toxicity of 4-S-CAP. Consistent with the known specificity of SSAO, the α-methyl analogue of 4-S-CAP did not appear to be a substrate.

MD 220661

This oxazolidinone compound is the primary amine derivative of the *R*- enantiomer (MD 240928) of the racemic compound MD 780236 (Figure 4.15). It behaves as a substrate for SSAO, whereas the corresponding *S*-enantiomer (MD 220662) appears to be a simple reversible inhibitor of the enzyme (Dostert *et al*. 1984).

Allylamine

This unsaturated amine is used in the manufacture of pharmaceuticals and vulcanised rubber. Prolonged exposure to allylamine can result in severe necrotic tissue damage with the cardiovascular system being particularly susceptible (Boor and Hysmith 1987). These toxic effects are not due to allylamine (3-aminopropene) itself but result from its metabolism to acrolein by SSAO (Nelson and Boor 1982; Hysmith and Boor 1988; see Figure 4.16). Allylamine is not a substrate for monoamine oxidase and its toxicity in the rat is greatly reduced by pretreatment with semicarbazide (Awasthi and Boor 1993). The hydrogen peroxide generated in the oxidation of allylamine by SSAO also appears to contribute to its toxicity and the addition of catalase to vascular smooth muscle cells *in vitro* partially protects against the toxicity (Ramos *et al.* 1988). Glutathione *S*-transferases catalyse the first step in the detoxification of acrolein (He *et al*. 1999). However, acrolein also activates glutathione *S*-transferase by binding to thiol groups and, although this appears to activate its own detoxification, the resulting glutathione depletion can, in turn, impair the individual's ability to detoxify other xenobiotics and reduce the capacity to remove hydrogen peroxide.

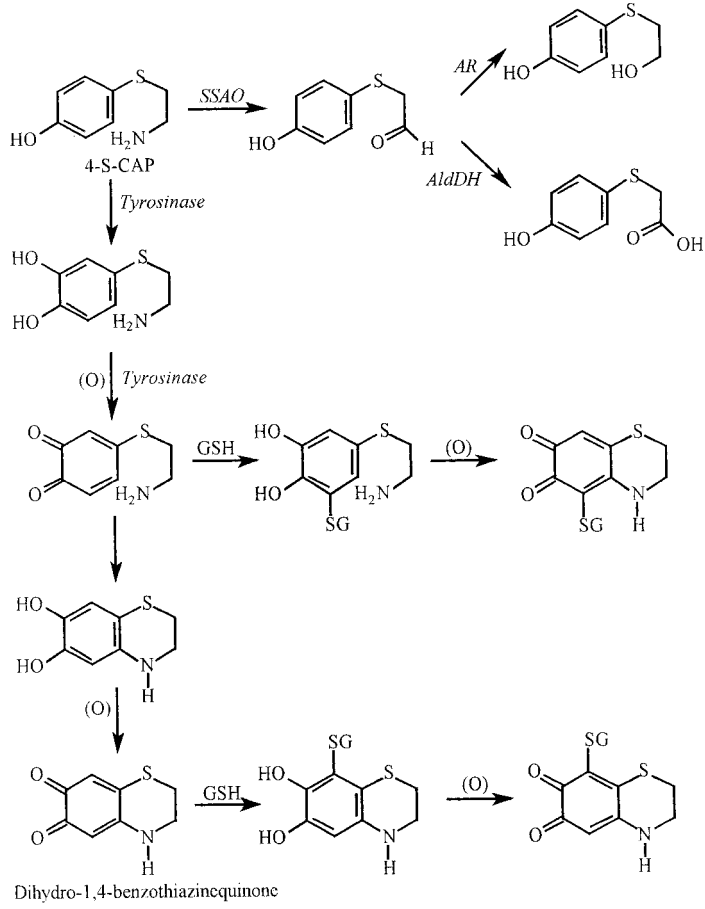


Figure 4.17 Metabolism of the melanocytotoxin 4-*S*-cysteaminylphenol (4-S-CAP). Generation of reactive oxygen radicals, during the autoxidation reactions and the quinone dihydro-1,4-benzothiazinequinone are believed to contribute most significantly to the cytotoxicity. The aldehyde produced through the action of SSAO is also cytotoxic.

WR-1065

WR-1065 (2-(3-aminopropylamino)ethanethiol) is a cytoprotective drug that is used to protect tissues against the damaging effects of radiation and some anticancer drugs. It is administered as the thiophosphate derivative amifostine (Ethyol; WR-2721; *S*-2-(3-aminopropylamino)ethyl-phosphorothioate) from which it is formed by the action of alkaline phosphatase (Shaw *et al.* 1996). It is converted by a 'copper-containing amine oxidase', present in the calf serum used for cell culture, to cysteamine and acrolein (Meier and Issels, 1995 see Figure 4.16). The reaction, which

was shown to be accompanied by oxygen consumption and hydrogen peroxide formation, was inhibited by the SSAO inhibitor aminoguanidine. Purified preparations of bovine plasma SSAO were also shown to catalyse the oxidation of WR-1065. The prodrug amifostine was shown not to be a substrate for this reaction. The oxidation of WR-1065 was shown to result in cytotoxicity and depletion of reduced glutathione. The precise details of the reaction are, however, unclear since the oxidative cleavage of WR-1065 would be expected to lead to the formation of cysteamine plus the aminoaldehyde, 3-aminopropanal, and the mechanism of the conversion of this compound to acrolein remains to be clarified. The involvement of one or more additional steps in acrolein formation would be consistent with the very low yield of this compound, relative to that of cysteamine, reported by Meier and Issels (1995). The major pathways of WR-1065 inactivation *in vivo* are the formation of the disulphide between two WR-1065 molecules, and mixed disulphides between WR-1065 and glutathione and cysteine, although cysteamine formation has also been detected (Shaw *et al*. 1996).

Tresperimus

Tresperimus ([4-{(3-aminopropyl)amino]butyl}carbamic acid,2-[[6-[(aminoiminomethyl)-amino]hexyl]amino]-2-oxoethyl ester) is an immunosuppressive drug that is metabolised in blood plasma rather than in the liver (Figure 4.18). The metabolic pathways involved have been studied by Claud *et al.* (2001) and shown to involve the SSAO-catalysed oxidation of the terminal amino group to the corresponding aldehyde, which may then be further oxidised to the carboxylic acid. The aldehyde can also decompose, non-enzymically, to the desaminopropyl derivative of tresperimus ([4-amino-butyl]carbamic acid,2-[[6-{(aminoiminomethyl)amino}hexyl]amino]-2-oxoethyl ester), which is also a substrate for SSAO giving an aldehyde, which is then further oxidised to the corresponding carboxylic acid. The involvement of SSAO in these processes was confirmed in the rat *in vivo* by its inhibition by hydralazine and in rat and human plasma *in vitro* by its inhibition by semicarbazide and by hydralazine. There was no significant role for polyamine oxidase (see below) in this process, but a minor role for MAO was not excluded. Although the production of the aldehyde derivatives takes place in the plasma, their further oxidation appeared to be a result of intracellular aldehyde dehydrogenase activity. Analysis of the urinary excretion patterns indicated that hydrolytic cleavage of the amide bond in the middle of the molecule to yield guanidinohexylamine (GHA) plus 2-[[[[4-[(3-aminopropyl)-amino]butyl]amino]carbo-nyl]oxy]-acetic acid (Figure 4.18) also occurred. This appeared to be a minor pathway in the human but was more important in the rat, which has very low plasma SSAO activities. There was no indication that oxidative deamination of GHA occurred.

DIAMINE OXIDASE

Although histamine is a poor substrate for some plasma SSAO enzymes, other diamines are not substrates for it (Buffoni 1966). However, there is a specific tissue-bound SSAO, diamine oxidase (DAO), which is found in high levels in the intestine,

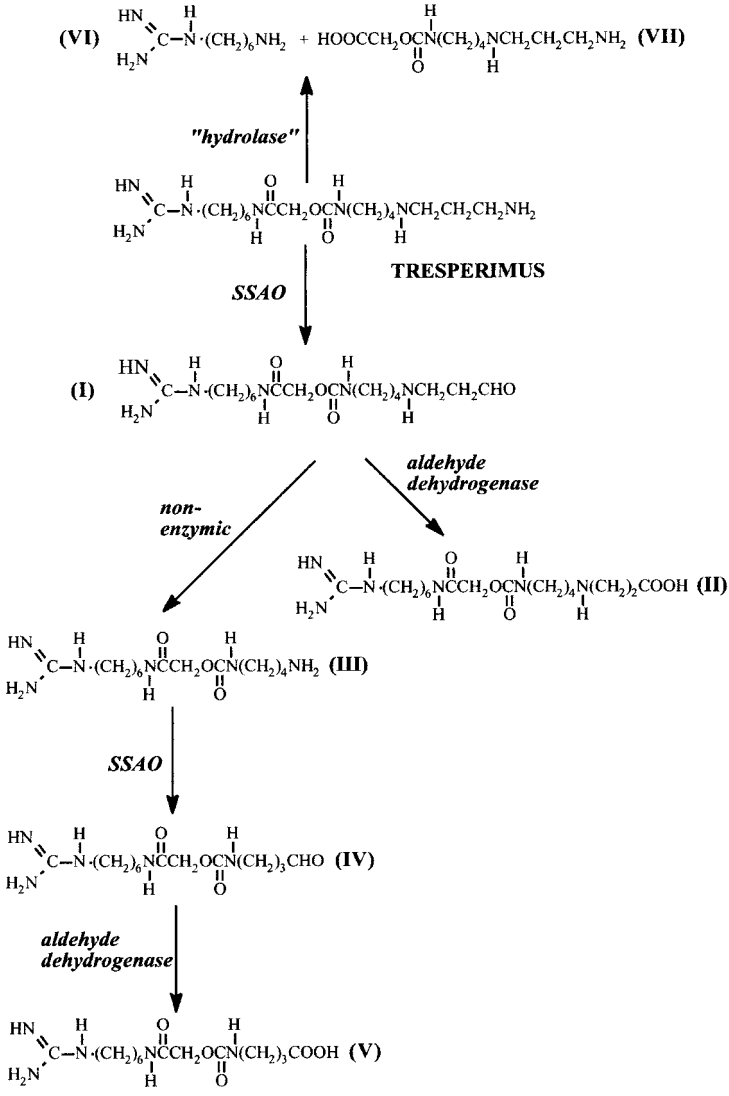


Figure 4.18 Metabolism of the immunosuppressive drug tresperimus ([4-{(3-aminopropyl) amino]butyl}carbamic acid,2-[[6-[(aminoiminomethyl)amino]-hexyl]amino]-2-oxoethyl ester). The products shown are: **(I)** [4-[(3-oxopropyl)-amino]butyl]carbamic acid,2-{[6-[(amino-iminomethyl)amino]hexyl]- amino}-2-oxoethyl ester); **(II)** 1-amino-1-imino-10,13-dioxo-12-oxa-2,9,14,19-tetraazadocosan-22-oic acid; **(III)** [4-aminobutyl]carbamic acid,2-[[6-{(aminoimino-methyl)amino}-hexyl]amino]-2-oxoethyl ester, (desaminopropyl- tresperimus); **(IV)** [4-oxobutyl]-carbamic acid,2-[[6-{(aminoiminomethyl)amino}hexyl]amino]-2-oxoethyl ester; **(V)** 1-amino-1-imino-10,13-dioxo-12-oxa-2,9,14-triazaoctadecan-18-oic acid; **(VI)** *N*-(6-aminohexyl)-guanidine (guanidinohexylamine; GHA); **(VII)** 2-[[[[4-[(3-aminopropyl)amino] butyl]amino]-carbonyl]oxy]-acetic acid.

kidney, thymus gland and placenta (Buffoni 1966), that has high activity towards histamine and other diamines, putrescine, cadaverine, spermidine and spermine. A problem in assessing its possible functions in the metabolism of xenobiotics is that it is not always clear whether work that describes an amine-oxidising activity that is inhibited by semicarbazide is referring to SSAO or DAO. However, agmatine (1-amino-4-guanidinobutane, Figure 4.19), has been shown to be a DAO substrate. Agmatine, which is an imidazoline receptor ligand (Holt and Baker 1995; Lortie *et al.* 1996), is produced from arginine by the action of arginine decarboxylase in plants and bacteria. More recently it has also been shown to be produced by rat brain, liver, and kidney (Lortie *et al.* 1996). It is present in some foods and beverages including, for example, beer (Izquierdo-Pulido *et al.* 1996) and, thus, may be regarded as a xenobiotic that is also produced endogenously. Several, but not all, amine oxidases are binding proteins for imidazoline and guanidine compounds (amiloride-binding proteins; Novotny *et al.* 1994; Lizcano *et al.* 1998) but only DAO appears to treat one of these, agmatine, as a substrate.

Polyamine oxidase

This enzyme (N^1-acetylspermidine: oxygen oxidoreductase (deaminating), E.C. 1.5.3.11; PAO) is a flavoprotein that is widely distributed in mammalian (rat and human) tissues, including brain and is also present in blood plasma (see Seiler 1995, 2000 for reviews). It oxidises a secondary amino group in monoacetylspermine and monoacetylspermidine to form, respectively, spermidine and putrescine (Hölttä 1977). The monoamine oxidase inhibitor pargyline is a relatively weak inhibitor of PAO. The drug MDL 72527, an *N,N′*-bis(2, 3-butadienyl)- derivative of putrescine (Figure 4.20), is a potent and selective inhibitor of PAO (Bey *et al.*1985). Rather surprisingly, PAO has been also reported to be inhibited by semicarbazide (Hölttä, 1977; Kunimoto *et al.* 1989).

The enzyme has been shown to oxidise the antimalarial drug MDL 27695, an *N,N′*-

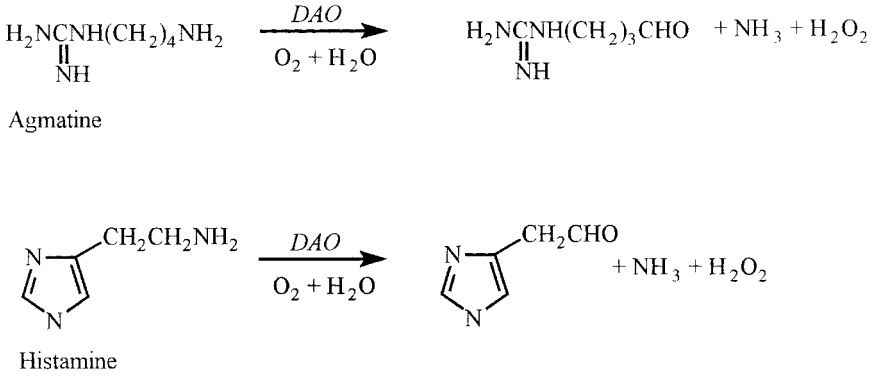


Figure 4.19 Oxidation of agmatine by diamine oxidase (DAO). The oxidation of histamine is also shown for comparison.

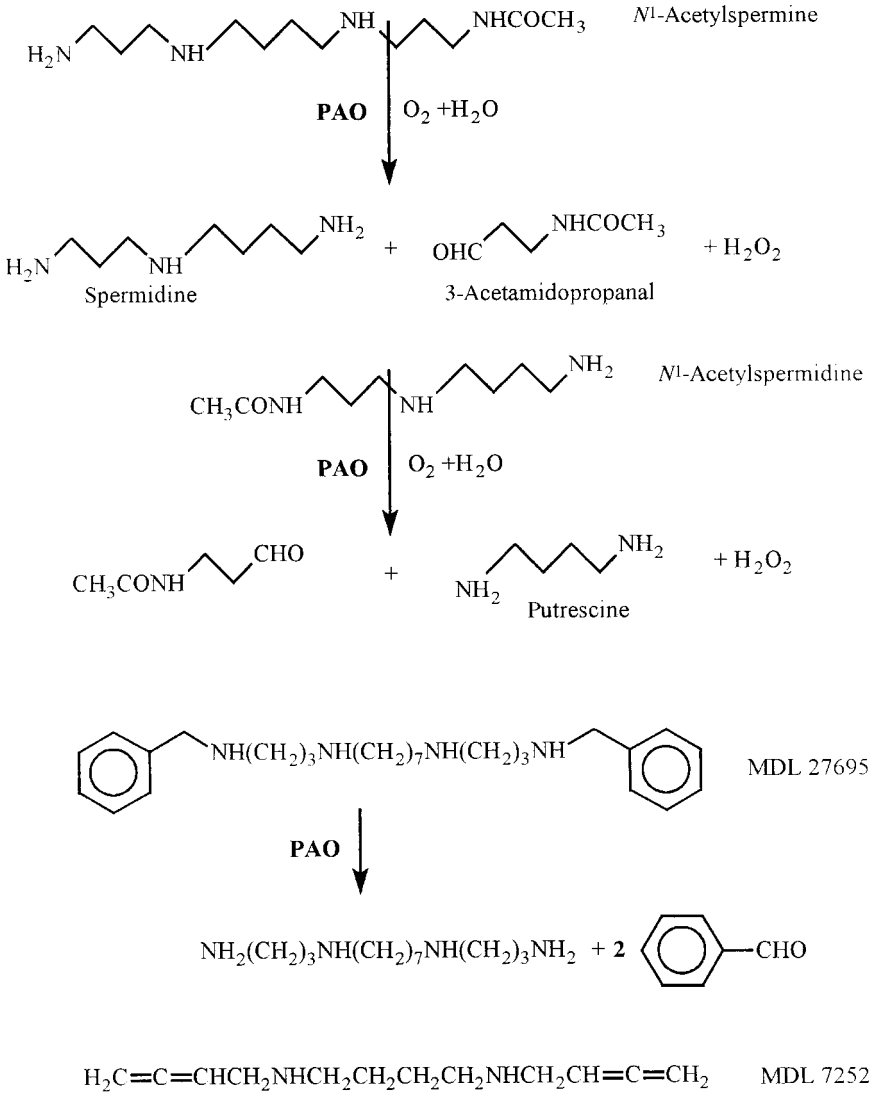


Figure 4.20 Some reactions catalysed by polyamine oxidase (PAO) and the structure of the PAO inhibitor MDL 72527.

bis(2, 3-benzylpolyamine)- derivative (Figure 4.20), which has also been shown to inhibit the growth of a rat hepatoma cell-line (Bitonti *et al.* 1989, 1990). Another cytotoxic agent *N*(1)-(n-octanesulphonyl)spermine is also oxidised by PAO and studies with the human colon carcinoma-derived CaCo-2 cell line have shown that oxidation serves to diminish its toxicity (Seiler *et al.* 2000).

An unusual reaction catalysed by PAO is the oxidative cleavage of milacemide (Strolin Benedetti *et al*. 1990, 1992b). However, the cleavage pattern is different from that catalysed by MAO in that glycinamide is not formed; the likely reaction products being pentylamine and oxamaldehyde (see Figure 4.13).

Conclusions

This chapter has concentrated on those xenobiotics which are known to be metabolised by the amine oxidases, at least *in vitro*. There are several other cases where too few data are available but it is possible that one or more of the amine oxidases may be involved in the metabolism of specific xenobiotics (for reviews see Dostert *et al*. 1989; Strolin Benedetti and Dostert 1994; Strolin Benedetti and Tipton 1998). Unfortunately we do not yet have sufficient information to be able to predict with certainty whether a compound will be a substrate for a specific amine oxidase in any given species (Wouters 1998). There have been too few metabolic studies to bridge the gap between *in vitro* studies and the behaviour in the, more complex, *in vivo* environment. Furthermore, the consequences that xenobiotic metabolism may have on the normal functions of these enzymes require more detailed study.

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