# Metabolic Bioactivation Reactions Potentially Related to Drug Toxicities

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The majority of metabolic transformations that xenobiotics undergo in mammalian systems leads to more polar and, in general, less pharmaco-logically and toxicologically potent products. This generalization has led to the proposal that evolutionary factors influencing the emergence of the principal enzymes responsible for the biotransformations of xenobiotics have a link to the improved survival potential of those individuals equipped with enzyme systems capable of converting otherwise toxic substances produced by plant- and animal-based food sources to nontoxic metabolites (Jakoby and Ziegler 1990).

The types of biotransformation reactions that many xenobiotics undergo have been organized into two principal classes: Phase I transformations that, for the most part, are oxidative in nature and generally introduce a hydroxy group into the substrate molecule; and phase II transformations that convert the newly introduced hydroxy group to polar conjugates such as glucuronides and sulfate esters (Caldwell 1986; Guengerich and Ziegler 1990). In the case of carboxylic acids, polar amido esters derived from various amino acids are formed (Killenberg and Webster 1980). Since many toxic natural products are lipophilic organic molecules, the metabolic conversion of these substances in land-bound animals to polar conjugates makes teleological sense in that polar compounds partition with difficulty across cell membranes but are readily filtered through the nephron and thus are more readily eliminated from the body through renal excretion. Depending on the structures of the molecules, however, the same metabolic events, on occasion, can generate chemically reactive and toxic metabolites (Mulder et al. 1986; Parke 1987; Korzekwa and Jones 1993; Guengerich 1994; Gonzalez and Gelboin 1994). Consequently, an important part of drug development focuses on the characterization of the metabolic profile of candidate drugs in an attempt to avoid structural features that may lead to the formation of toxic metabolites.

This chapter is concerned with the metabolic fate of cyclic tertiary amines and, in particular, a consideration of potential bioactivation

pathways that may lead to toxic metabolites. Cyclic tertiary amines form an important class of compounds in the area of drug abuse since a variety of psychoto-mimetic agents such as cocaine (figure 1, structure 1), lysergic acid diethylamide (LSD 25) (structure 2) and phencyclidine (structure 3), as well a large group of centrally acting compounds such as the neuroleptic phenothiazine chlorpromazine (structure 4), the central nervous system (CNS) stimulant mazindol (structure 5), the narcotic analgetic fentanyl (structure 6), and the antidepressant imipramine (structure 7) either have been used to treat CNS disorders and/or have the potential for abuse. These types of compounds undergo extensive oxidative metabolic transformations.

The discussion that follows focuses on selected examples of metabolic transformations that may be linked to the formation of potentially neurotoxic metabolites. Although the number of well-characterized examples of such biotransformations is relatively few, it may be reasonable to speculate that the neurological disorders associated with long-term exposure to substances of abuse and some behavior-modifying medications may involve biochemical lesions mediated by chemically reactive metabolites. Thus, it may be important when attempting to assess the possible significance of metabolic bioactivation processes to appreciate that the chemical instability of reactive metabolites which can make the identification and characterization of their biological properties difficult.

The most important metabolic transformation that cyclic amines undergo is -carbon oxidation which generates the corresponding iminium products (Koymans et al. 1993). This reaction is catalyzed by members of the cytochrome P450 superfamily of hemoproteins (Nelson et al. 1993). As discussed below, recent studies have documented that the outer mitochondrial membrane bound flavoproteins monoamine oxidase A and B (MAO-A and MAO-B) are efficient catalysts for the -carbon oxidation of a specific class of cyclic tertiary amines, namely 4-substituted 1-methyl-1, 2, 3, 6-tetrahydropyridines (Kalgutkar et al. 1995). The most generally accepted catalytic pathway (figure 2) for this reaction assumes an initial single electron transfer (SET) step from the amine substrate (structure 8) nitrogen lone pair to generate an aminyl radical cation (structure 9) which, following loss of an -proton, is converted to a highly reactive carbon-centered radical (structure 10). A second single- electron oxidation of the carbon-centered radical gives, in the case of the cytochrome P450-catalyzed reaction, the carbinolamine (structure 11),

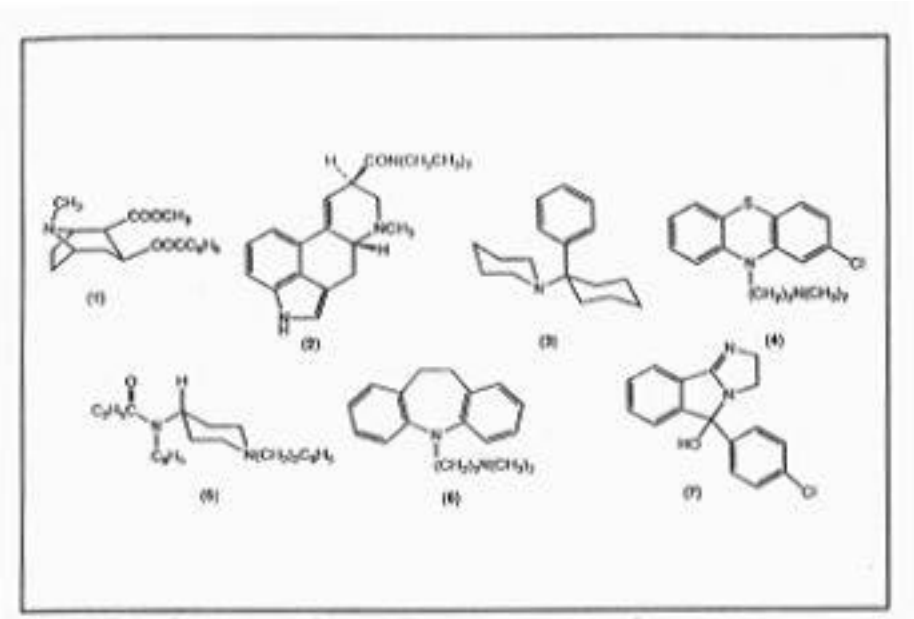

**FIGURE 1.** *Structures of centrally acting compounds.*

which is in equilibrium with the iminium ion product (structure 12). In the MAO-catalyzed reaction, intermediate structure 10 is converted directly to structure 12. As shown in figure 2, the electron acceptor for the cytochrome P450-catalyzed reaction is the perferryl oxo species ($Fe^{V}O$), while the electron acceptor for the MAO-catalyzed reaction is the oxidized flavin moiety (FAD).

Although the experimental evidence supporting the SET mechanism for the cytochrome P450-catalyzed (Miwa et al. 1983; Hanzlik et al. 1984; Guengerich and Macdonald 1984; Macdonald et al. 1989) and the MAO-catalyzed (Silverman 1992) reactions is extensive, it has been challenged by results from several laboratories. These include results obtained with deuterium kinetic isotope effects (Peterson et al. 1987; Peterson and Castagnoli 1988; Dinnocenzo et al. 1993; Walker and Edmondson 1994), model chemical reactions (Kim et al. 1993, 1995), and the unexpected substrate properties (Kuttab et al. 1994) of certain tertiary cyclopropyl- amine derivatives, that according to the SET pathway, would be expected to act as enzyme inactivators only (Silverman 1984). These results have led some investigators to propose a direct hydrogen atom abstraction pathway (structure 8 –> structure 12) that bypasses the aminyl radical cation intermediate (structure 9), for both the cytochrome P450-catalyzed (Dinnocenzo et al. 1993) and the MAO-catalyzed pathways (Walker and Edmondson 1994; Ottoboni et al. 1989), and a polar (2-electron)

pathway for MAO catalysis that proceeds via an amine-FAD adduct (Kim et al. 1993, 1995). The metabolic processes in figures 2 to 9 are described below.

As illustrated in figure 2, these -carbon oxidations lead to the formation of iminium ion products which undergo spontaneous hydrolysis to the corresponding aldehyde (structure 13) and secondary amine (structure 14),
the net outcome being N-dealkylation. Cyclic tertiary amines (figure 3, structure 15) also undergo oxidative N-dealkylation, via hydrolysis of the enzyme generated exocyclic iminium intermediate (figure 3, structure 16), to yield an aldehyde (structure 13) and the cyclic secondary amine (figure 3, structure 17). The corresponding oxidation of a ring -carbon atom generates the cyclic iminium intermediate (figure 3, structure 18). Unlike the acyclic regioisomer structure 16, hydrolysis of structure 18 to the aminoaldehyde structure 19 is reversible, giving rise to the possible further metabolic processing of structure 18. These intermediary metabolites are often oxidized to the biologically less active lactams (structure 20) in a reaction that is catalyzed by the liver cytosolic enzyme aldehyde oxidase (Bielawski et al. 1987). If special structural features are present in the substrate molecule or if the cyclic iminium metabolite is generated in extrahepatic tissues lacking aldehyde oxidase, these reactive

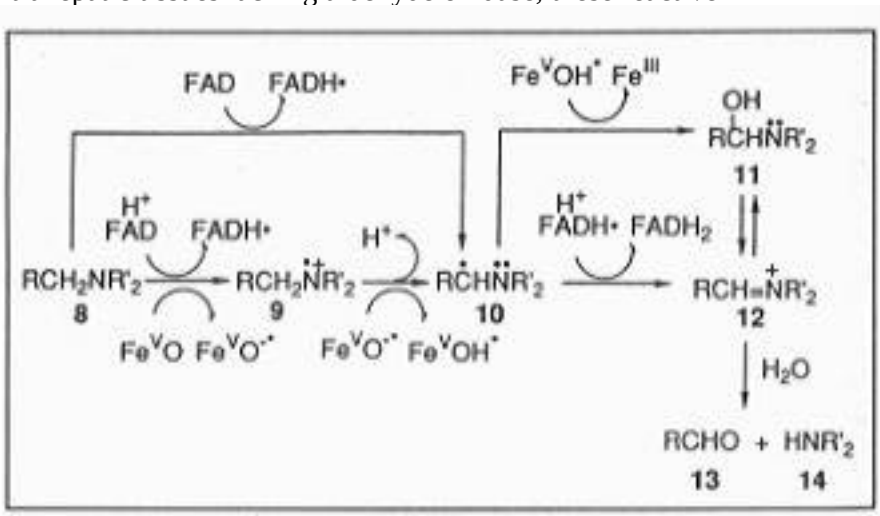


**FIGURE 2.** *Proposed pathways for the MAO-catalyzed and cytochrome P450-catalyzed oxidations of tertiary amines.*

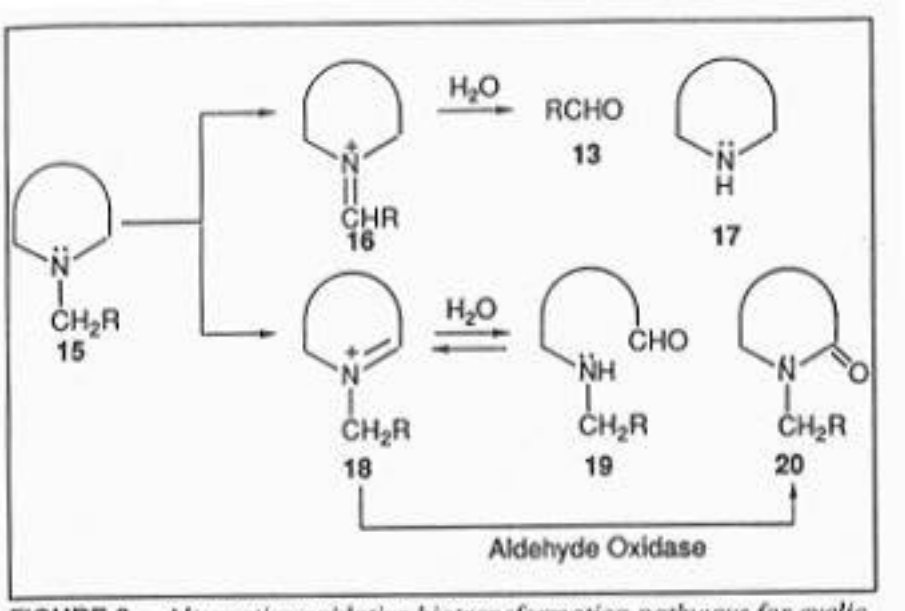


**FIGURE 3.** *Alternative oxidative biotransformation pathways for cyclic tertiary amines.*

intermediates may undergo alternative chemical transformations that can produce toxic products.

An example of the metabolic conversion of a 5-membered azaheterocylic system to toxic metabolites is summarized in figure 4 with the hepatotoxic and carcinogenic pyrrolizidine alkaloids (Mattocks 1986). Initial cyto-chrome P450-catalyzed, -carbon oxidation of the bicyclic parent tertiary amine (structure 21) generates the iminium intermediate (structure 22) that, upon loss of a proton, forms the pyrrolic derivative (structure 23). However, compound 23 is unstable because of the presence of the leaving groups in the side chains. Departure of the $RCOO^-$ group generates the highly reactive electrophilic intermediate (structure 24) that is attacked by a nucleophilic group present on biomacromolecules to generate an adduct (structure 25). The pyrrolizidine alkaloids are *bis*alkylating agents because of the presence of the second strategically positioned leaving group ($R.COO^-$) with the resulting formation of a crosslinked biopolymer (structure 26).

The dramatic toxicity of the pyrrolizidine alkaloids and related highly toxic compounds has led investigators to focus their attention on the

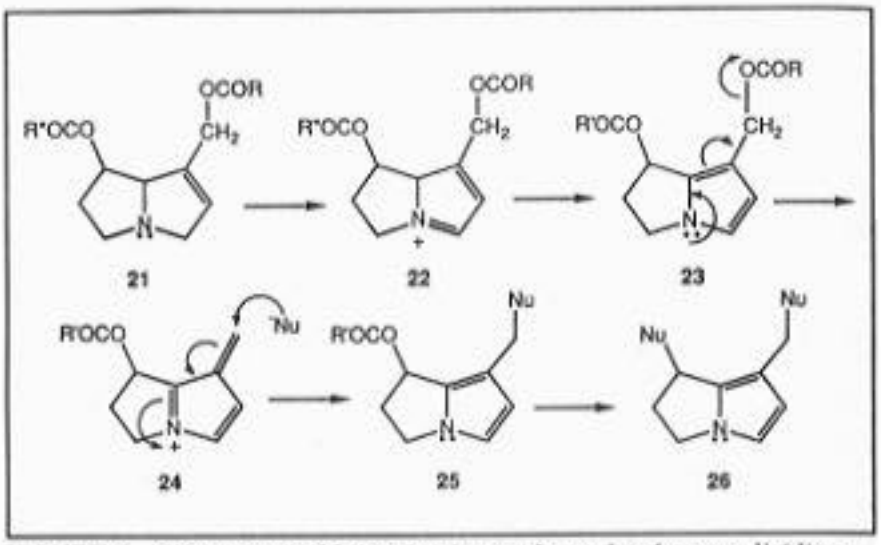


**FIGURE 4.** *The proposed bioactivation pathway for the pyrrolizidine alkaloids.*

underlying biochemical mechanisms. More subtle toxicities, however, may be associated with compounds that undergo similar oxidative conversions. The authors have been interested in the potential metabolic bioactivation of the tobacco alkaloid (S)-nicotine (figure 5, structure 27), a compound that is metabolically bioactivated to reactive intermediates that form covalent bonds to biomacromolecules (Shigenaga et al. 1988). The principal oxidative pathway for this compound also proceeds via cytochrome P450-catalyzed oxidation to form the corresponding iminium metabolite (structure 28). When generated in the presence of liver aldehyde oxidase, structure 28 is rapidly converted to the nontoxic lactam (S)-cotinine (structure 29). In the absence of aldehyde oxidase, however, the iminium ion (structure 28), presumably via the corres-ponding free enamine base (structure 30), can be oxidized to the pyrrolic metabolite -nicotyrine (structure 31) in a reaction that is catalyzed by MAO-B (Shigenaga 1989). -nicotyrine is an electron-rich heterocyclic aromatic system that undergoes rapid cytochrome P450-catalyzed conver- sion to the pyrrolinones (structures 35 and 36), which in turn autoxidize to the 5-hydroxypyrrolinone (structure 38) (Shigenaga et al. 1989).

The proposed reaction pathway leading to these products is depicted in figure 5. The electron-rich pyrrole ring system is oxidized to the reactive arene oxide (structure 32) which rearranges to the zwitterionic species

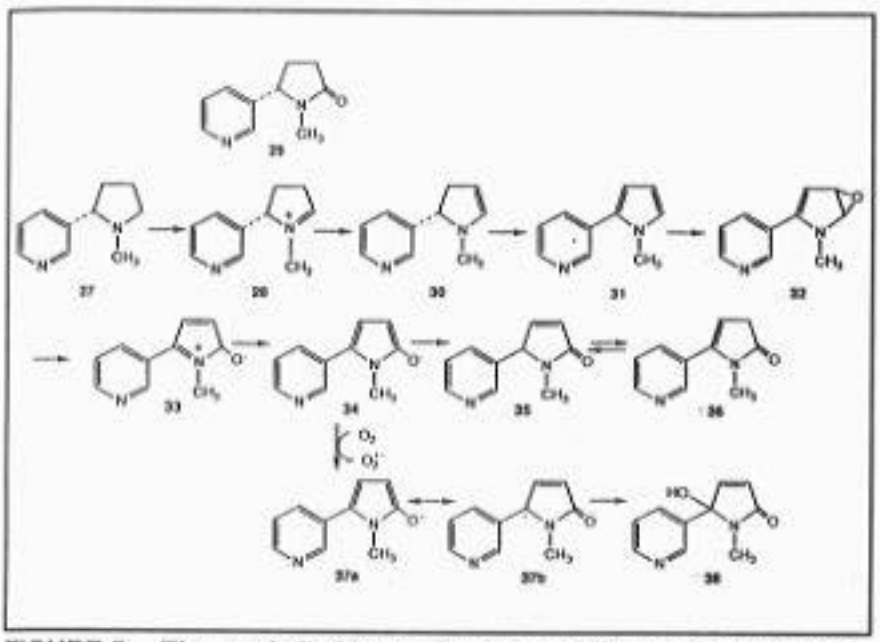

**FIGURE 5.** *The metabolic biotransformation of (S)-nicotine leading to potentially toxic metabolites.*

(structure 33). Proton loss generates the anion structure 34 which leads to an equilibrium mixture of pyrrolinones (structures 35 and 36). Alternatively, the anion (structure 34) undergoes autoxidation, leading to formation of superoxide radical anion ($O_2^{\cdot -}$) and the resonance stabilized radical, structure 37a <—> 37b, which eventually is converted to the final product (structure 38). The possible toxicological significance of this metabolic pathway remains to be documented. Since human exposure to tobacco products occurs over the course of many years, even low-level exposures to reactive intermediates (such as structure 37) could have a cumulative effect that may contribute to the degenerative processes linked to tobacco use. In this regard, the efficient conversion of (S)-nicotine to the corresponding iminium metabolite (Shigenaga et al. 1988) by lung cytochrome P450 may be particularly significant since the levels of aldehyde oxidase in this tissue are likely to be very low or absent (Huff and Chaykin 1967; Beedham 1985).

A major impetus for considering the bioactivation of cyclic tertiary amines is derived from studies on the parkinsonian-inducing nigrostriatal neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) structure 39. Extensive metabolic,

biochemical, and toxicological investigations have documented that the neurodegenerative properties of MPTP are mediated by a mitochondrial neurotoxin that is formed according to the reaction sequence shown in figure 6 (Sayre 1989; Maret et al. 1990; Kopin 1992; Tipton et al. 1993; Tipton and Singer 1993; Singer et al. 1993). The substrate amine (structure 39) undergoes C-6 (allylic) ring -carbon oxidation to form the cyclic iminium metabolite, the 1-methyl-4-phenyl-1,2-dihydropyridinium species ($MPDP^+$) (structure 40). Structure 40, although stable as its solid perchlorate salt (Chiba et al. 1985), is too unstable to isolate from incubation mixtures (Weissman et al. 1985). Since structure 40 is an excellent substrate for aldehyde oxidase, it is rapidly converted in whole liver homogenates to the corresponding lactam structure 41 (Wu et al. 1988). In the absence of the aldehyde oxidase, however, structure 40 (< 50 millimolars (mM)) in pH 7.4 buffer undergoes slow autoxidation to yield the 1-methyl-4-phenyl-pyridinium product $MPP^+$ depicted in structure 42 (Wu et al. 1988). At higher con- centrations, this dihydropyridinium metabolite also may participate in two alternative reactions (figure 7). The first is a bimolecular dispropor-tionation reaction in which the free base structure 43 derived from structure 40 functions as a hydride donor, while structure 40 serves as a hydride acceptor. The net result is the formation of stoichiometric amounts of MPTP and $MPP^+$ (Wu et al. 1988). A second and more complex reaction sequence involves the net consumption of three moles of the dihydropyridinium metabolite (structure 40) that eventually yields the isoquinoline system (structure 44), a mole of $MPP^+$, and a mole of methyl- amine (figure 7) (Leung et al. 1989). The extent to which these or similar reactions involving endogenous reactants occur in vivo is not known.

In vitro metabolic studies with rodent and human liver microsomal prepara- tions have established that MPTP undergoes both oxidative N-demethylation and C-6 (allylic) oxidation in reactions that are -nicotinamide adenine dinucleotide phosphate (NADPH) dependent and therefore likely to be cytochrome P-450 catalyzed (Weissman et al. 1985; Ottoboni et al. 1990). Although the latter transformation can lead to the toxic pyridinium metabolite $MPP^+$, the cytochrome P450-catalyzed pathway is unlikely to contribute significantly to the neurotoxicity of MPTP. As mentioned above, liver aldehyde oxidase diverts the inter-mediate dihydropyridinium metabolite away from pyridinium ion formation by catalyzing the conversion of structure 40 to the nontoxic lactim structure 41. Further-more, even if formed in the periphery, the polar pyridinium metabolite would have limited access to the central nervous system (CNS). The low

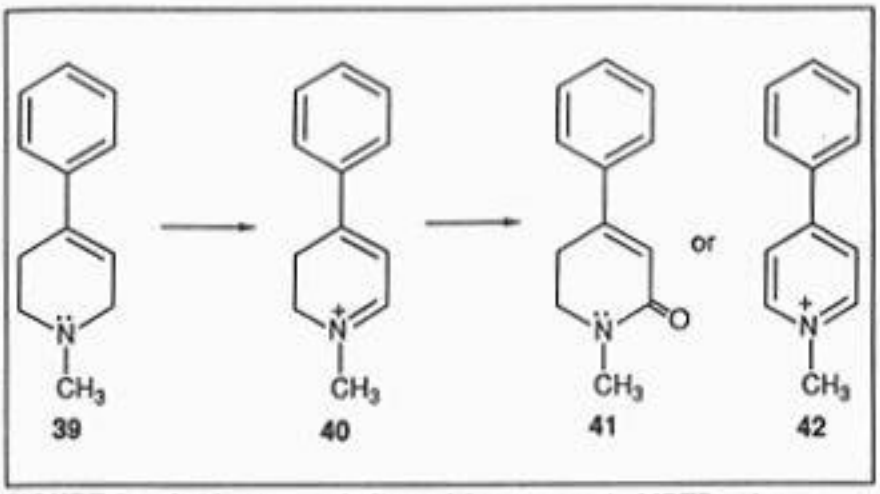


**FIGURE 6.** *Oxidative metabolism of the neurotoxin MPTP to its dihydropyridinium metabolite MPDP⁺ and subsequent detoxification by conversion to a dihydropyridone or oxidative toxication to the ultimate toxic species MPP⁺.*

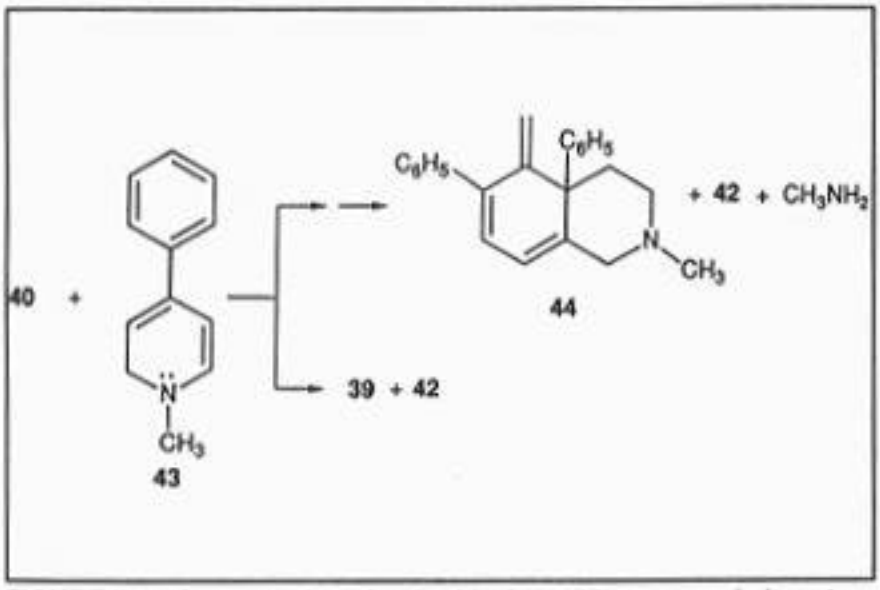


**FIGURE 7.** *Alternative fates for the dihydropyridinium metabolite of MPTP.*

concentrations of the P450s in the brain (Warner et al. 1993) preclude the in situ formation of toxic levels of $MPP^+$ within the CNS by this pathway.

Continued interest in the possible metabolic activation of MPTP led to brain tissue homogenate studies that initially established bioactivation in these tissues by MAO (Chiba et al. 1984). Later studies demonstrated the unexpected and excellent ($k_{cat}/K_M$ = 1400 $min^{-1}mM^{-1}$ at 37%C) MAO-B substrate properties of MPTP (Kuttab et al. 1994). Subsequent studies employing a monkey model of MPTP-induced parkinsonism established the role of MAO-B in the mediation of the nigrostriatal toxicity of MPTP. The critical experiment showed that the selective MAO-B inhibitor (R)-deprenyl (structure 45, figure 8) completely protects against MPTP's toxicity in this model (Langston et al. 1984; Heikkila et al. 1984). MPTP also is a substrate for MAO-A ($k_{cat}/K_M$ = 47 $min^{-1}mM^{-1}$ at 30%C) (Singer et al. 1986). However, since pretreatment with the MAO-A selective inactivator clorgyline (structure 46) does not protect against its neurotoxicity, this form of the enzyme does not appear to contribute to MPTP's neuro-degenerative properties.

The selective toxicity of MPTP is remarkable, particularly since there is no evidence for the presence of MAO-B in the susceptible dopaminergic nigrostriatal neurons (which do, however, contain MAO-A) (Moll et al. 1990). This apparent dilemma has been resolved by the demonstration that $MPP^+$ is a substrate for the dopamine transporter (Javitch et al. 1985). Once localized intraneuronally, $MPP^+$ is concentrated further within the inner mitochondrial membrane (Youngster et al. 1989*a*; Davey et al. 1992), where it inhibits electron transport (Nicklas et al. 1985, 1987) leading to adenosine triphosphate (ATP) depletion (Di Monte et al. 1986) and cell death.

The ability of MPTP to cause a lesion that parallels in many ways the characteristic lesion of idiopathic Parkinson's disease has stimulated efforts to identify possible environmental and endogenous compounds that may possess MPTP-type properties (Ikeda et al. 1992). Intracerebral microdialysis studies that estimate irreversible neuronal degeneration have provided evidence that a variety of pyridinium and related quaternized azaheteroaromatic systems are toxic to dopaminergic neurons (Rollema et al. 1990, 1994). Nevertheless, relatively few compounds meet all of the characteristics required for

an MPTP-type neurotoxin; these characteristics include the in situ MAO-B-catalyzed biotransformation in the brain to a pyridinium

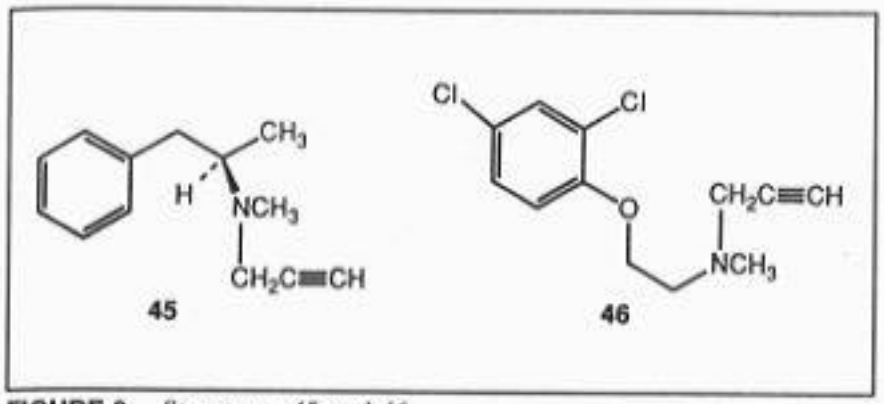


**FIGURE 8.** *Structures 45 and 46.*

metabolite. The metabolite is actively transported into the nigrostriatal nerve terminals and then into the inner mitochondrial membrane, where it must inhibit electron transport. Extensive studies have documented that only 1-methyl-1,2,3,6- tetrahydropyridine derivatives bearing selected substituents at C-4 are good substrates for MAO-B (Maret et al. 1990; Sablin et al. 1994; Youngster et al. 1989*b*; Kalgutkar et al. 1994). Furthermore, various types of tetrahydropyridine derivatives that are good substrates for MAO-B do not display MPTP-type activity when tested in vivo since, for various structural reasons, the intermediate dihydropyridinium metabolites are not converted to the corresponding pyridinium species (Naiman et al. 1990; Dalvie et al. 1992).

The MAO-catalyzed bioactivation of MPTP to $MPDP^+$, leading to the neurotoxic $MPP^+$ pyridinium metabolite, is dependent on the allylamine unit present in the tetrahydropyridine moiety. A similar reaction sequence, however, may occur with piperidine derivatives lacking the double bond but at the same oxidation state as the tetrahydropyridine. One compound of particular interest that fits this description is haloperidol (figure 9, structure 47), a potent neuroleptic agent that, like other members of this pharmacological class, causes severe extrapyramidal side effects including parkinsonism and tardive dyskinesias (Tarsy and Baldessarini 1986). This 4-piperidinol derivative resembles MPTP in that it bears an aryl group at C-4 of the piperidinol. Dehydration of HP structure 47, a reaction that is reported to occur in microsomal incubations (Fang and Gorrod 1991), gives the corresponding 1,2,3,6-tetrahydropyridine derivative HPTP, structure 50. HP 47 and HPTP 50 are not substrates for MAO-B, but

evidence obtained with the aid of mass spectral techniques and a sensitive high-performance liquid chromatography (HPLC) fluorescence assay have documented the conversion of HP (in humans) and both HP and HPTP (in rodents) to the pyridinium product $HPP^+$ (structure 52) (Subramanyam et al. 1990, 1991*a*, 1991*b;* Igarashi and Castagnoli 1992; Van der Schyf et al. 1994). The proposed metabolic sequence for the oxidation of HPTP to $HPP^+$ (figure 9) proceeds via the dihydropyridinium intermediate structure 51 followed by autoxidation of structure 51 to structure 52 (Subramanyam et al. 1991*b*). The oxidation of HP is thought to proceed via initial formation of the iminium inter-mediate structure 48 which, via the aminoenol structure 49, is converted to the dihydropyridinium species structure 51. These ring -carbon oxidations parallel the pathway outlined in figure 3 for cyclic tertiary amines in general. Since oxidative N-dealkylation (analogous to the sequence 8 –> 12 –> 13 + 14, figure 2) is a major metabolic pathway for HP (Forssman and Larsson 1977), it is not surprising to observe ring - carbon oxidation as a competing pathway.

The metabolic pathways leading to the production of these urinary pyridinium metabolites are likely to be mediated by one or more forms of liver cytochrome P450. In vitro metabolic studies with rodent (Igarashi et al., unpublished results) and human (Usuki et al., submitted) microsomal preparations have demonstrated the NADPH-dependent oxidation of both HP and HPTP to $HPP^+$. Ongoing studies in the authors' laboratory have shown that $HPP^+$ and related pyridinium metabolites are present in brain tissues obtained from C57 black mice that had been treated with HPTP (Van der Schyf et al. 1994). Additionally, results obtained from intra-cerebral microdialysis, mitochondrial respiration, and rat embryonic mesencephalic cell culture studies suggest that $HPP^+$ possesses $MPP^+$ type neurotoxic properties (Rollema et al. 1992, 1994; Bloomquist et al. 1994).

The critical question concerns the neurotoxic potential of HP in the human. Since the development of drug induced tardive dyskinesias often requires months or even years of drug exposure (Gerlach and Casey 1988; Casey 1991), the demonstration of toxin-induced lesions in experimental animals may be difficult. Furthermore, in view of the dramatic species selectivity of MPTP (Singer et al. 1987; Giovanni et al. 1994*a*, 1994*b*), the absence of a detectable anatomical lesion in HP- or HPTP-treated rodents may not provide a definitive answer to the question

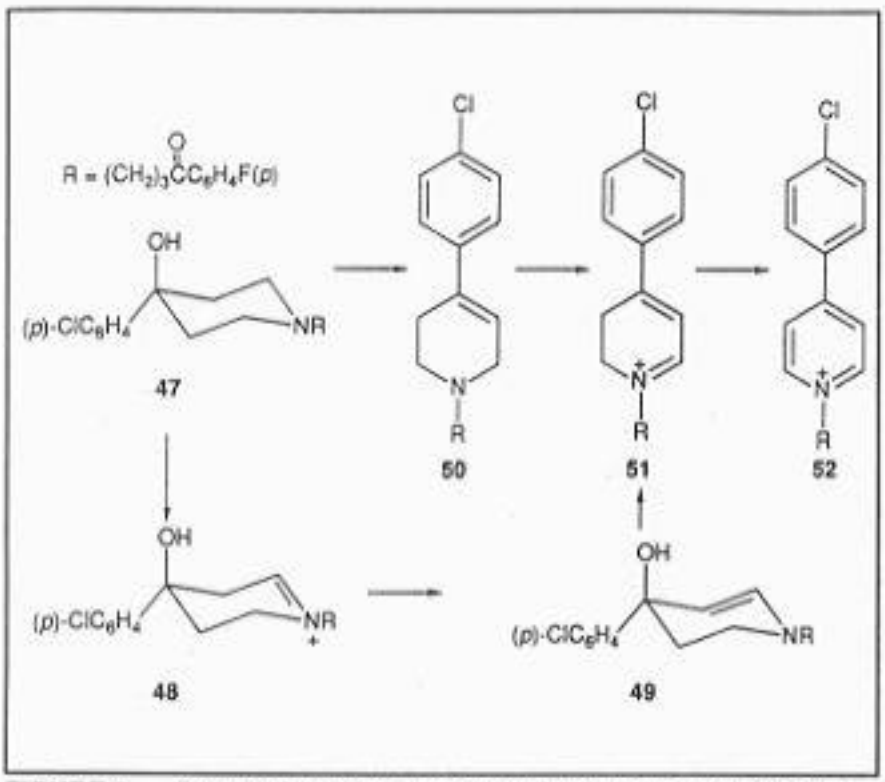


**FIGURE 9.** *Proposed metabolic pathways leading to the pyridinium metabolite HPP⁺.*

of the neurotoxic potential of HP. It is hoped that this issue will be resolved by an ongoing study in baboon (Van der Schyf et al., unpublished observations).

The potential neurotoxicity of pyridinium and related quaternary azaheterocyclic species and the possible formation of such metabolites from six-membered azaheterocyclic systems are of interest because of the many drugs, including many CNS-acting agents, that contain such structural features. Reports on the metabolism of compounds such as LSD (structure 3) morphine (structure 4), phencyclidine (structure 5), and fentanyl (structure 6) have not provided evidence for pyridinium ion formation. However, the proposed metabolites are quaternary cations and might not be readily detected without appropriate analytical tools. Investigations of the possible bioactivation of cyclic tertiary amines to neurotoxic quaternary cationic azaheterocyclic metabolites could provide valuable information that might lead to a better understanding of the

drug-related CNS disorders associated with long-term exposure to certain abused substances.

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