The Role of Iminium-Enamine Species in the Toxication and Detoxication of Cyclic Tertiary Amines

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INTRODUCTION AND BACKGROUND

Aliphatic cyclic tertiary amines constitute a major class of naturally occurring and synthetic drugs directed at central biogenic amine receptors. Microsomal metabolism of these amines is known to be associated with low levels of covalent binding and/or suicide inactivation of the pertinent metabolizing P-450 isozymes; two of the more notorious examples are phencyclidine (1-(1-phenylcyclohexyl)piperidine) (PCP) (Hoag et al. 1984) and nicotine (Shigenaga et al. 1988).

Covalent binding was initially believed to result from alkylation of protein-based nucleophiles by the respective endocyclic iminium metabolite arising from cytochrome P-450 mediated two-electron oxidation (Hoag et al. 1984; Nguyen et al. 1979; Overton et al. 1985; Shigenaga et al. 1988; Ward et al. 1982). These iminium species are generated in equilibrium with a carbinolamine, an endocyclic enamine, and a ring-opened aminocarbonyl compound (figure 1). In contrast to *acyclic* tertiary amines, where the iminium intermediates are readily hydrolyzed via carbinolamines to secondary amines and aldehydes (or ketones) (figure 2), iminium hydrolysis for *cyclic* tertiary amines remains reversible, ensuring the persistence of all equilibrium species in figure 1. This explains why covalent binding is seen only for cyclic tertiary amines.

Support for the notion that the iminium electrophiles could be the covalent binding species is found in their ready formation of stable cyanide adducts and the fact that the presence of cyanide during metabolism of the parent amines protected against covalent binding (Hoag et al. 1984; Nguyen et al. 1979; Shigenaga et al. 1988; Ward et al. 1982). However, cyanide is a special nucleophile (it forms a C-C bond) that may not be representative of most physiologic

nucleophiles. Also, although nicotine-$^{1.(5.)}$-iminium forms a p-methylthiophenol adduct (Brandange and Lindblom 1979*a*), the iminium does not bind to nucleophilic polyamino acids except polycysteine, and this only in the presence of O_2 (Obach and Van Vunakis 1988). Unpublished studies from the authors' laboratory indicate that simple cyclic iminium species form stable covalent adducts with typical bionucleophiles only under special conditions, and no evidence has been obtained for the persistent binding of iminium species directly under physiologic conditions.

The protection against covalent binding by cyanide trapping of the initial iminium metabolite could be equally well explained if a species derived from metabolism beyond the iminium stage were the responsible culprit. In recent years, increasing evidence has arisen that most covalent binding does in fact arise from some type of *advanced* metabolite (Hoag et al. 1984, 1987; Osawa and Coon 1989; Sayre et al. 1991). For example, recent studies on the independently synthesized PCP iminium demonstrate little (Hoag et al. 1987) or no (Osawa and Coon 1989) P-450 inactivation in the absence of NADPH (the reduced form of nicotinamide-adenine dinucleotide phosphate). If the iminium species itself is not directly responsible for covalent binding, then it becomes possible to explain why glutathione (GSH) and related thiols can protect against metabolism-dependent covalent binding even though their iminium adducts only form reversibly (Hoag et al. 1984; Obach and Van Vunakis 1988; Ward et al. 1982), viz, GSH is evidently trapping the advanced reactive metabolite(s).

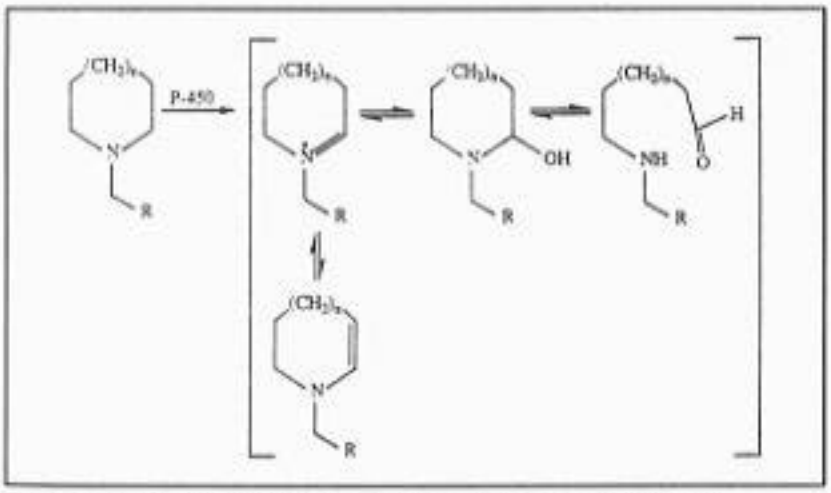


FIGURE 1. *Endocyclic N-dealkylation remains a reversible equation.*

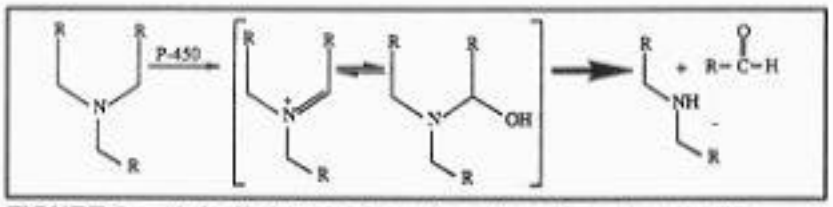


FIGURE 2. *N-dealkylation of acyclic amines is an irreversible process.*

-Aryl-substituted amines such as PCP and nicotine possess pK_as about 1 unit lower than normal tertiary amines, and enamines have pK_as about 1 unit lower than the corresponding amines (Cook 1988). Thus, cyclic enamines corresponding to PCP and nicotine, for example, should have pK_as of about 8.5, suggesting that the enamines could reach a concen- tration of nearly 10 percent that of the iminium at physiologic pH. Although higher pH also favors the carbinolamine (Brandange and Lindblom 1979*a*), the enamine would be favored over the more hydrophilic carbinolamine in a biological compartment of low dielectric constant such as the cytochrome P-450 active site. Such biological compartment will not only favor enamine relative to carbinolamine, but will also decrease the effective iminium pK_a on account of favoring charge minimization. In fact, the enamine appears to be the form of the iminium that elutes under reverse phase chromatographic conditions (Hallstrom et al. 1983; Herber et al. 1991; Mattammal et al. 1987).

The electron-rich enamines should be excellent substrates for processing by enzymes involved in oxidative metabolism, and could be giving rise to second-generation metabolites with reactive properties. A potentially important role of endocyclic enamines in xenobiotic metabolism has not been widely appreciated. It is proposed here that the enamine, rather than the ring-opened aminoaldehyde, is the iminium-derived species giving rise to metabolism-dependent P-450 inactivation. This hypothesis suggests that there should be a correlation between the degree of inactivation and the tendency of various heterocyclic ring sizes to exist in ring-closed versus ring-opened form. Such correlation is supported by a study on analogs of PCP, which revealed a rank order of $6 > 5 >> 7$ in ring size for loss of both benzphetamine demethylase activity and heme CO binding (Brady et al. 1987). As expected, N,N-diethylphenylcyclohexylamine, the acyclic version of the 5-membered ring PCP analog, exhibited no significant loss of heme.

Detoxication at the endocyclic iminium stage of metabolism is traditionally considered to involve iminium conversion to lactams by the action of the cytosolic molybdoenzyme aldehyde oxidase (AO), the prototype reaction for which is the conversion of pyridinium compounds to 2- and 4-pyridones (Felsted et al. 1973). As far as xenobiotics are concerned, the best example has been the conversion of nicotine $^{1.(5.)}$- iminium to the major human metabolite cotinine (figure 3) (Brandange and Lindblom 1979*b*). Some additional examples of lactam formation have been reported in the literature (Hammer et al. 1968; Lin et al. 1993; Wall and Baker 1989). However, although the generality of this transformation has been assumed, a consistent detoxication of iminium species by this pathway has never been demonstrated. In the key case of PCP, no lactam metabolite analogous to cotinine has ever been reported.

A preliminary report by Obach and Van Vunakis (1990) claimed that cotinine could also be formed by a microsomal nicotinamide adenine dinucleotide $(NAD)^{+}$-dependent dehydrogenase (abbreviated MND). Inhibitor studies suggested that MND is not a typical aldehyde dehydrogenase. The presence of this activity in rabbit microsomes was confirmed in the authors' laboratories (Flammang 1994). The rate of cotinine production from the $^{1.(5.)}$ -iminium by this route was found to be comparable to the rate of conversion of nicotine to the $^{1.(5.)}$ -iminium (as assessed by this enzyme might play in xenobiotic metabolism in general. It is expected that the substrate-structure dependence of MND will be quite different from that of cytosolic AO.

Detoxication at the iminium/enamine/carbinolamine stage also occurs through oxidation and reduction of the ring-opened aminoaldehyde by aldehyde and alcohol dehydrogenases, respectively, which have wide subcellar distribution. Significant differences are not expected in metabolic rates for various acyclic aldehydes arising from carbinolamine dissociation; detoxication via such routes should depend mainly on the equilibrium concentration of the ring-opened as opposed to ring-closed forms, and overall may play a minor role. Overall, the iminium/enamine stage of metabolism (figure 1) is viewed by the authors as the critical determinant between toxic activation and detoxication. Inefficient conversion to lactams by cytosolic AO and/or MND appears to correlate with increasing levels of covalent binding.

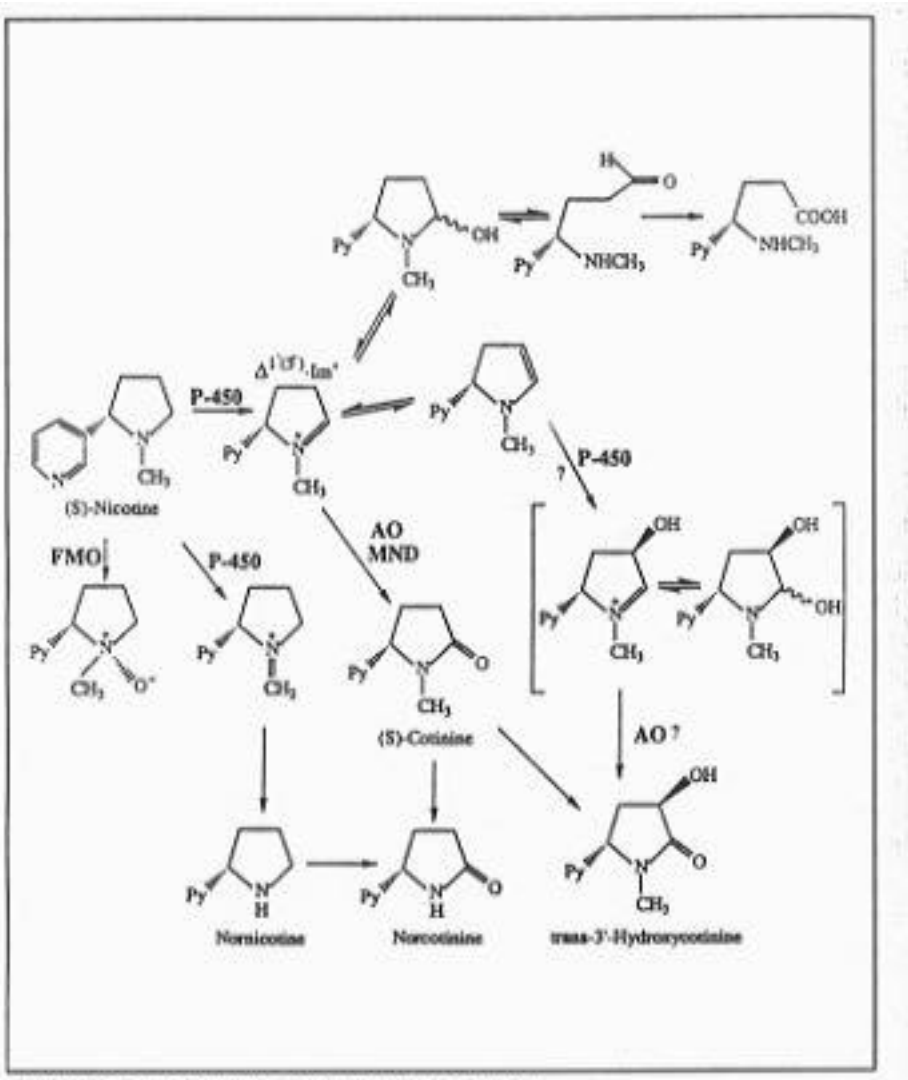


FIGURE 3. *Pathways of nicotine metabolism.*

KEY: AO = aldehyde oxidase; FMO = flavin monooxygenase; MND =microsomal NAD^+-dependent dehydrogenase.

In an effort to elucidate the nature of endocyclic iminium-derived species that give rise to metabolism-dependent covalent binding and to clarify what factors govern the balance between toxic activation and detoxication

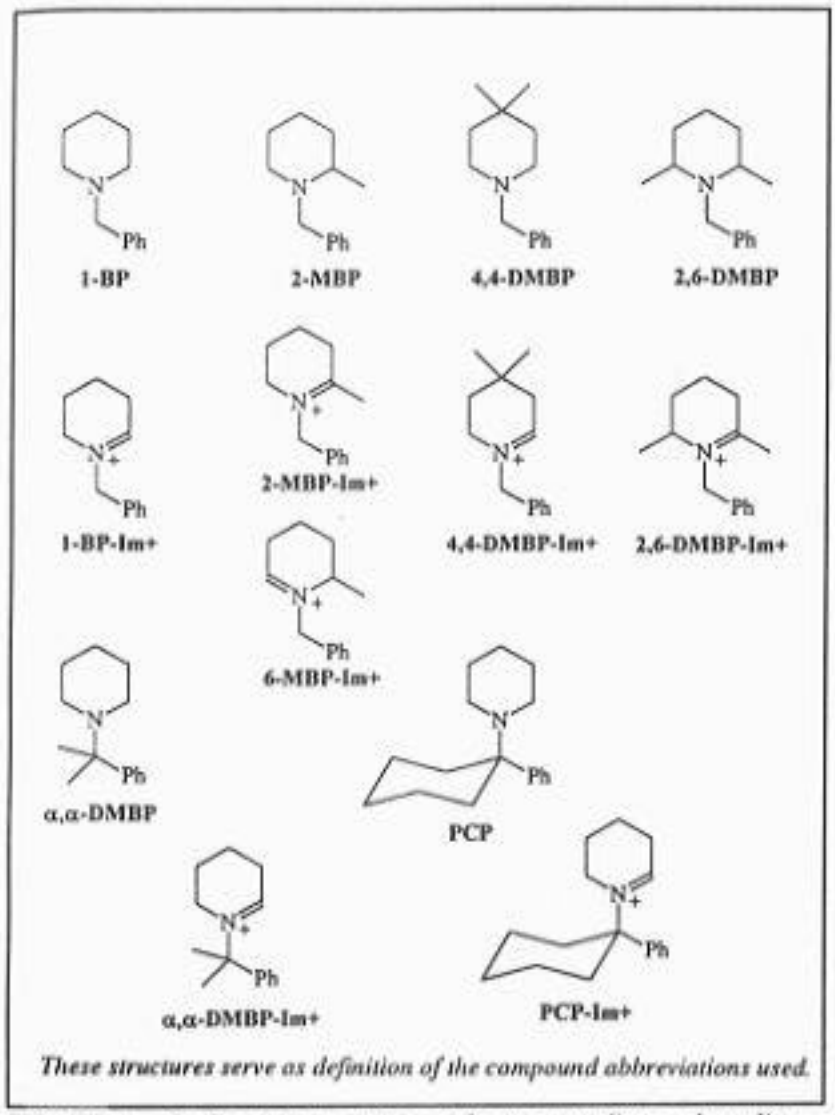


FIGURE 4. *Cyclic tertiary amines and corresponding endocyclic iminium metabolites investigated in this study.*

at the iminium stage of cyclic tertiary amine metabolism, the authors initiated a series of studies to examine the effects of systematic structural changes at the level of simple piperidine and pyrrolidine derivatives as prototypes for PCP and nicotine, respectively. These compounds permit a focus on nitrogen-directed metabolism and the role of the iminium

species, since other metabolic pathways are eliminated. For example, study of 1-benzylpiperidine (1-BP) as a structurally abbreviated form of PCP would give information about metabolic toxication/detoxication uncomplicated by the numerous cyclohexyl ring-derived PCP metabolites (Gole et al. 1988; Holsztynska and Domino 1985). The expectation that 1-BP would exhibit the endocyclic metabolism of interest and not just N-debenzylation was based on the report of metabolism-dependent covalent binding for its homolog, 1-benzylpyrrolidine (Ho and Castagnoli 1980).

Using phenobarbital-induced rabbit liver microsomes and an NADPH regenerating system, data was obtained on metabolic rates and products, cytochrome P-450 inactivation, and the effect of $CN^{å}$ trapping on these parameters (Engelhart 1994). The methods have been described in an initial report (Sayre et al. 1991), and are not duplicated here. Synthetic procedures for the amines and iminium compounds (figure 4) are described separately.

RESULTS AND DISCUSSION

Metabolic Profiles of Parent Amines

The metabolic rates and major product profiles for the tertiary amines studied are shown in table 1. The first three amines listed undergo oxidation principally at the N-C bond, leading to the endocyclic (major) and exocyclic (minor) iminium intermediates, the latter dissociating to a secondary amine (not quantified) and benzaldehyde. The preference for encyclic over exocyclic oxidation was also seen previously for 1-benzyl-pyrrolidine (Ho and Castagnoli 1980). Exocyclic N-dealkylation is blocked in the case of PCP and ,-DMBP. Cytochrome P-450 binding constants obtained for 1-BP, 2,6-DMBP, and PCP were 92, 37, and 10 micromolars (M), respectively, and appear to reflect the differences in metabolic rates and the concentrations needed to observe maximal metabolism. Although PCP was oxidized more rapidly than 1-BP and 2,6-DMBP, the fraction of metabolism resulting in formation of the PCP-Im^{+} was lower, consistent with the fact that PCP additionally gives rise to cyclohexyl ring-derived metabolites. Interestingly, 4,4-DMBP exhibited a higher rate than did 1-BP and 2,6-DMBP, but a smaller fraction of the total metabolism was accounted for by the corresponding iminium. For 1-BP, 1-BP-Im^{+} 1 was not observed directly, but the THA dimer 3 was observed instead (figure 5). Dimer 3 is the expected

TABLE 1. *Substrate activity of tertiary amines for cytochrome P-450 using microsomes from phenobarbital-induced rabbits.*

Substrate	Metabolic rate (nmol/min/mg)	Major products formed during initial metabolic phase[a]
1-BP	55 (10 mM)	C -C .dimer (50%), PhCHO (1.5%)
1-BP (1mM KCN)	52 (10 mM)	-cyano (25%), PhCHO (1.0%)
1-BP-THA dimer	40 (10 mM)	
2,6-DMBP	65 (10 mM)	iminium (71%), PhCHO (0.5%)
2,6-DMBP (1mM KCN)	59 (10 mM)	-cyano (58%), PhCHO (0.5%)
2-MBP	54 (10 mM)	iminium C -C . dimer, PhCHO[b]
4,4-DMBP	112 (10 mM)	iminium (12%), PhCHO (3.2%)
4,4-DMBP (1mM KCN)	31 (10 mM)	PhCHO (7.1%)
4,4-DMBP-Im$^+$	44 (1 mM)	
PCP	89 (1 mM)	iminium (30%)
PCP (1mM KCN)	80 (1 mM)	-cyano (11%)
PCP-Im$^+$	80 (1 mM)	
,-DMBP	37 (1 mM)	iminium (89%)
,-DMBP (0.5 mM KCN)	42 (1 mM)	iminium (37%), -cyano (50%)
,-DMBP-Im$^+$	70 (1 mM)[c]	

KEY: a = Represented as percentage of the total starting material consumed; b = yields not quantified because the authors did not distinguish between the two possible iminiums and the various possible C -C .dimers; the PhCHO was found in only trace amounts, however. c = estimated as a lower limit. PhCHO = benzaldehyde; KCN = potassium cyanide; THA = tetrahydro-anabasine. Other abbreviations are per figure 4.

outcome of iminium-enamine C -C . coupling (Leonard and Hauck 1957). Control studies on the authentic iminium perchlorate 1 established the short lifetime of this species under the incubation conditions (pH 7.4, 37%C). The endocyclic iminium could, however, be trapped as the cyano adduct 4 when metabolism was conducted in

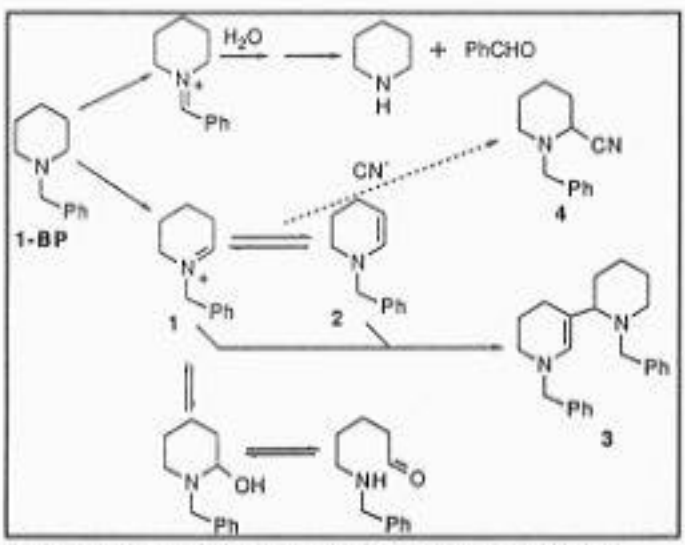


FIGURE 5. *P-450 metabolism of 1-benzylpiperidine.*

the presence of 1 mM KCN (no dimer 3 was detected in this case). For the other amines, where ring methyl substitution was expected to sterically inhibit iminium-enamine coupling (Leonard and Hauck 1957), the corresponding iminiums were observed directly. Control studies on the independently prepared iminium salts (figure 4) established their relatively long-term stability under both high performance liquid chromatography (HPLC) assay and incubation conditions, with the exception of 6-MBP-Im^+, which is analogous to 1-MBP-Im^+. On account of the instability of 6-MBP-Im^+, no attempt was made to distinguish the isomer distribution of the iminium metabolite arising from 2-MBP. As previously reported in the case of PCP and 1-benzylpyrrolidine (Hoag et al. 1984; Ho and Castagnoli 1980; Ward et al. 1982), the iminium metabolites could be trapped as the -cyano adducts in the presence of 1 mM cyanide, though this trapping was incomplete in some cases.

P-450 Inactivation

Data on the ability of various amines and metabolic intermediates to inhibit microsomal benzphetamine demthylase activity are listed in table 2. This is the standard assay for the phenobarbital-inducible P-450

TABLE 2. *Inhibition of benzphetamine N-demethylase activity.*

Substrate (1 mM)	% activity direct assay	% activity after pelleting/resuspension
1-BP, - G6PD	99[b]	99 Å 2
1-BP, + G6PD	57 Å 5	65 Å 7
1-BP, + G6PD (0.5 mM KCN)	73 Å 4	77 Å 3
2-MBP, - G6PD	99 Å 1	105 Å 3
2-MBP, + G6PD	57 Å 5	58 Å 2
2-MBP, + G6PD (0.5 mM KCN)	75 Å 9	81 Å 2
2-MBP-Im^+, - G6PD	96 Å 3	101 Å 3
2-MBP-Im^+, + G6PD	85 Å 3	89 Å 8
6-MBP-Im^+, - G6PD	81 Å 4	
6-MBP-Im^+, + G6PD	43 Å 3	
2,6-DMBP, -G6PD	98[b]	98 Å 3
2,6-DMBP, + G6PD	71 Å 3	80 Å 2
2,6-DMBP-Im^+, - G6PD	96 Å 4	108 Å 2
2,6-DMBP-Im^+, + G6PD	54 Å 2	63 Å 1
4,4-DMBP, - G6PD	95 Å 2	104 Å 3
4,4-DMBP, + G6PD	85 Å 11	93 Å 4
4,4-DMBP, + G6PD (0.5 mM KCN)	83 Å 11	86 Å 4
4,4-DMBP-Im^+, - G6PD	99[b]	102 Å 3
4,4-DMBP-Im^+, + G6PD	75 Å 6	86 Å 3
PCP, - G6PD	98[b]	104 Å 3
PCP, + G6PD	58 Å 6	57 Å 9
PCP, + G6PD (0.5 mM KCN)	78 Å 5	88 Å 4
PCP-Im^+, - G6PD	80 Å 2	78 Å 4
PCP-Im^+, + G6PD	36 Å 2	27 Å 1
,-DMBP, - G6PD	95 Å 3	104 Å 3
,-DMBP, + G6PD	43 Å 3	45 Å 5
,-DMBP, + G6PD (0.5 mM KCN)	75 Å 2	75 Å 5
,-DMBP-Im^+, - G6PD	91 Å 7	97 Å 4
,-DMBP-Im^+,+ G6PD	37 Å 4	46 Å 3

KEY: a = Incubated for 30 minutes, 37%C, pH 7.4 with microsomes from PB-induced rabbits. Values are percent of control activity Å S.E.M. of at least 3 determinations; b = Single determination; c = 0.5 mM concentration.

isoform (2B4) that is the most active in aliphatic amine metabolism. No inhibition by the parent tertiary amines was seen in the absence of metabolism (the - G6PD data). However, all parent amines exhibited a metabolism-dependent inactivation of cytochrome P-450 that was predominantly irreversible, as shown by the data obtained after pelleting/resuspension of the microsomes. The rank order of inhibitory potency was ,-DMBP > PCP ~ 2-MBP > 1-BP > 2,6-DMBP > 4,4-DMBP. The authors' interpretation of the increased level of metabolism-dependent inactivation seen for PCP and ,-DMBP relative to 1-BP is that removal of the exocyclic C-N dehydrogenation pathway (1-BP metabolism gives substantial N-debenzylation) results in an increased endocyclic metabolism, which apparently is the source of enzyme inactivation. In fact, for ,-DMBP, the level of inactivation is virtually the same as that seen for the parent amine, implying that essentially all parent amine is metabolized through endocyclic C-N dehydrogenation. The finding that ,-DMBP-Im $^{+}$and PCP-Im^{+} are equipotent inactivators confirms that the weaker inactivation of PCP relative to ,-DMBP at the parent amine stage reflects the alternate ability of PCP to be metabolized on the cyclohexyl ring.

If the iminium species is on the pathway of metabolism-dependent inactivation of P-450 by the parent amine, the independently prepared iminium should be a more potent inactivator. In an earlier study, no attempt was made to evaluate the P-450 inactivating potential of 1-BP-Im^{+} because of its rapid "dimerization" to 3 (Sayre et al. 1991). However, the iminium species corresponding to the parent amines 2,6-DMBP, 4,4-DMBP, and PCP were evaluated directly and were found to exert a greater inhibitory potency than the parent amines. The finding that most (see below) of the increased inhibition was dependent on the presence of G6PD (which again was not reversed by pelleting/resuspen-sion) indicates that the main inactivating potential arises not from the iminiums themselves but from their further metabolism.

In the case of 2-MBP, the finding that the more substituted iminium 2-MBP-Im^{+} is a weaker inactivator than the parent amine, whereas the less substituted iminium 6-MBP-Im^{+} is a more potent inactivator, indicates that endocyclic C-N metabolism on the less-substituted side is primarily responsible for inactivation by the parent amine.

The finding that PCP-Im^{+} elicits significant irreversible inactivation in the a absence of G6PD (a similar result was obtained for 6-MBP-Im^{+}) is consistent with the earlier report by Hoag and colleagues

(1987) suggesting a possible direct binding of this iminium to the enzyme. However, the G6PD-independent inactivation of the iminiums may represent residual NADPH in the microsomal preparations, because Osawa and Coon (1989) observed essentially no G6PD-independent activity loss for PCP-Im^+ using purified rabbit P-450 forms 2, 3b, and 6 in a reconstituted system.

Based on the ability to trap, at least partially, the various iminium metabolites using cyanide in the incubation medium, it was expected that cyanide would inhibit the loss of cytochrome P-450 activity if the iminium species were an obligatory intermediate in the inactivation process. As indicated in table 2, partial protection in the presence of 0.05 mM (or less) KCN was observed in the case of 1-BP, 2-MBP, ,-DMBP, and, consistent with earlier findings (Hoag et al. 1984), PCP. However, the presence of higher concentrations of KCN (1 mM and above) resulted in either no apparent protection against inactivation or an actual enhancement of inactivation by the parent amines (data not shown). The latter finding is contrary to the expectation that higher [CN'] would result in more efficient trapping of the iminium species, and even at [CN'] = 1 mM, trapping of a ,-DMBP-Im^+ (table 1) and especially 2,6-DMBP-Im^+ (not shown) was incomplete. The apparent discrepancies in the cyanide protection experiments may be explained in part by the observation that the corresponding -cyano adducts (independently synthesized) are themselves rather potent inhibitors of the enzyme (data not shown). Another complicating factor is that 1 mM KCN causes a significant degree of inhibition of benzphetamine demethylase activity in its own right. Further work will be needed to arrive at a fully satisfactory explanation for the cyanide results.

Enamine-Derived Metabolites

Evidence for oxidative metabolism of the endocyclic enamines has been obtained for both 1-BP and PCP. In a previously reported (Masumoto et al. 1991) preparative scale metabolism of 1-BP, it was found that in addition to the C -C . coupled dimer 3, small amounts of 1-benzyl-3- piperidol (1-BP-3-ol), 1-benzyl-4-piperidol (1-BP-4-ol), and 1-benzyl-3-piperidone (1-BP-3-one) were obtained (figure 5). It was further found that cyanide reduced greatly the levels of 1-BP-3-one metabolite but not the 1-BP-3-ol metabolite, suggesting that 1-BP-3-one is generated principally from the initial metabolite (1-BP-Im^+) rather than from oxidation of 1-BP-3-ol (Masumoto et al. 1991; Sayre et al. 1991). Consistent with this notion is the finding of relatively large amounts of the corresponding 3-one in the

microsomal metabolism of PCP under conditions where the 4-ol is observed exclusively of any 4-one (Masumoto et al. 1989). A plausible mechanism for cytochrome P-450 metabolism of endocyclic enamines consistent with the generation of 3-piperidones is shown in figure 6.

Also shown in figure 6 is an alternate route of enamine metabolism that would lead to a 2-en-4-ol and, after subsequent oxidation, to a 2-en-4-one. Although no 2-en-4-one was detected in the microsomal metabolism of 1-BP (Sayre et al. 1991), the corresponding 2-en-4-one was found by Hoag and colleagues (1988) as a major product resulting from microsomal metabolism of PCP-Im^+. Also of interest is the report by Zhao and colleagues (1991) that exposure of PCP-Im^+ to rat brain and liver mitochondria resulted in the isolation of a 3-formyl derivative that can be rationalized in terms of formylation of PCP-enamine by a tetrahydrofolic acid formyl donor. These iminium/enamine metabolic pathways for PCP are summarized in figure 7. At this time, the information available on the fate of metabolism of the endocyclic enamines derived from 1-BP and PCP does not permit an assessment of the actual species responsible for covalent binding.

Detoxication

As mentioned above, detoxication at the enamine/iminium/carbinolamine stage occurs in large part through oxidation to a lactam by both a cystosolic AO and a newly discovered MND. Although nicotine-$^{1.(5.)}$-iminium is readily converted to cotinine by both enzymes, the lack of any reported lactam metabolite in the case of PCP suggests that the exocyclic -branching in this case was sterically interfering with oxidation at the endocylic - position. Using the rabbit liver postmicro- somal supernatant as source of AO, it was confirmed that PCP-Im^+ and ,-DMPB-Im^+ are not converted to lactam (2-one) metabolites (independently synthesized). However, additional preliminary studies indicate that even some sterically unencumbered iminium species are poor substrates for AO (see table 3). The substrate structure-activity factors which control iminium metabolism by this enzyme have never been thoroughly investigated, and deserve further research. One obvious factor may be the effect of ring size on the equilibrium between iminium, carbinolamine, and ring-opened aldehyde forms.

As far as metabolism by MND is concerned, no substrate other than nicotine $^{1.(5.)}$-iminium has been identified to date. Evidence that at

least some interaction with the enzyme can be obtained, however, is indicated by the ability of at least one iminium species to partially inhibit the conversion of nicotine [1.(5.)]-iminium to cotinine (table 3). Nonetheless, general iminium ion metabolism is certainly not the case.

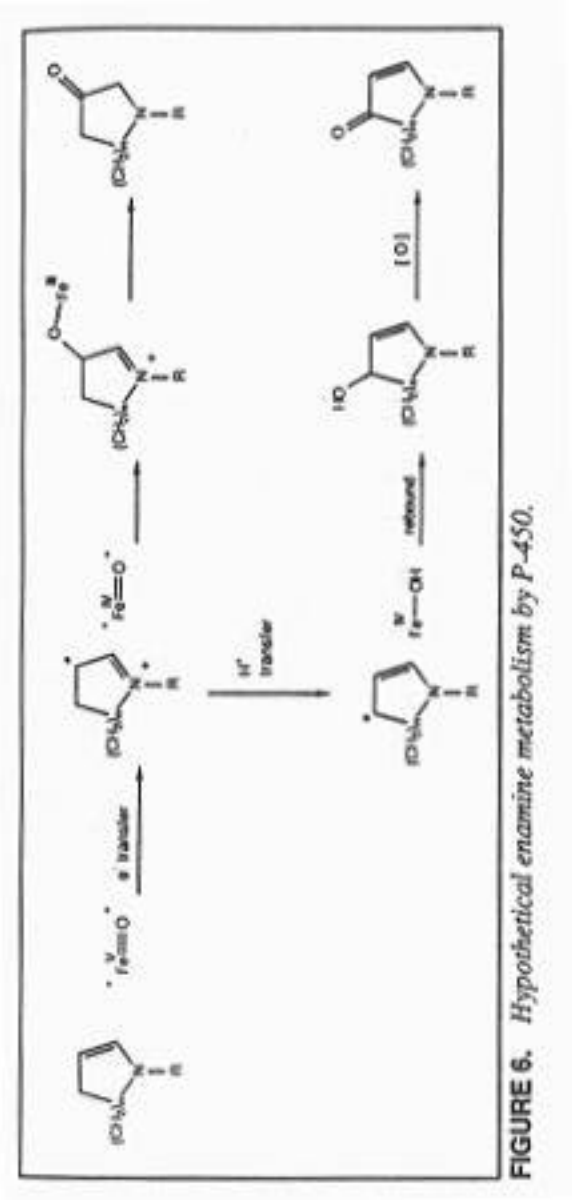


FIGURE 6. *Hypothetical enamine metabolism by P-450.*

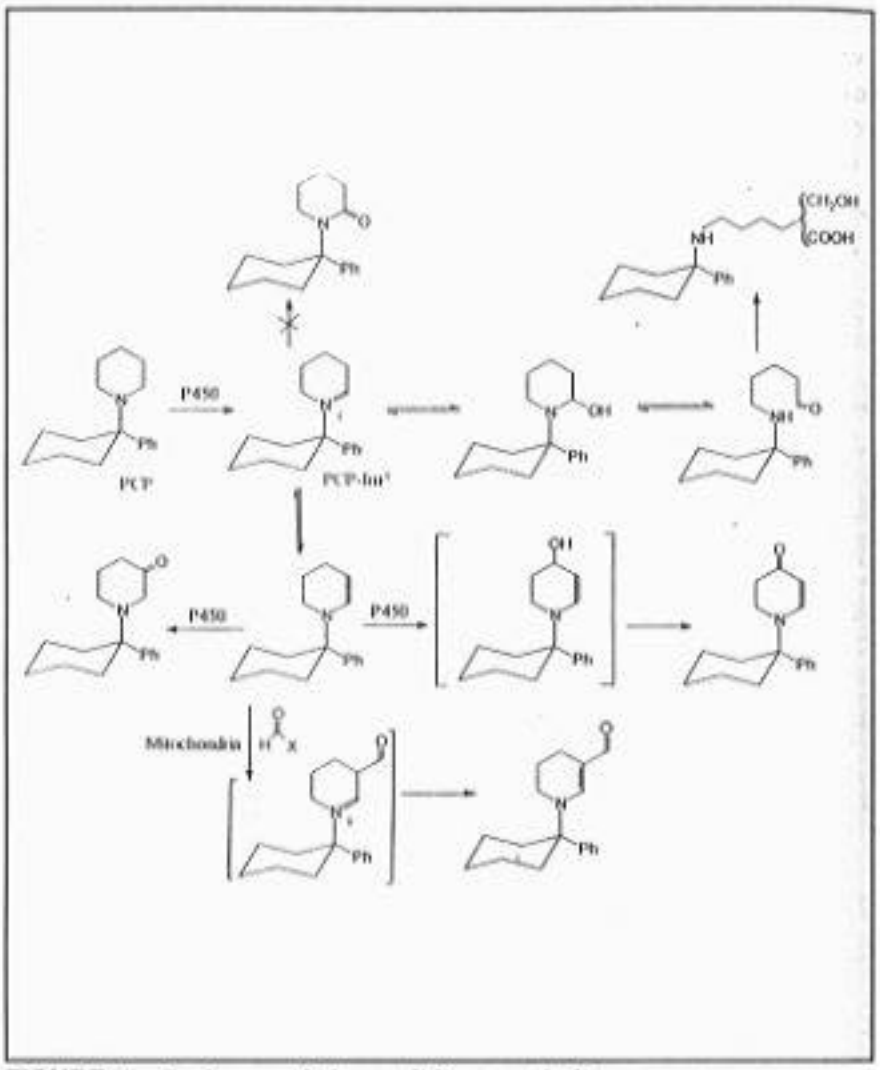
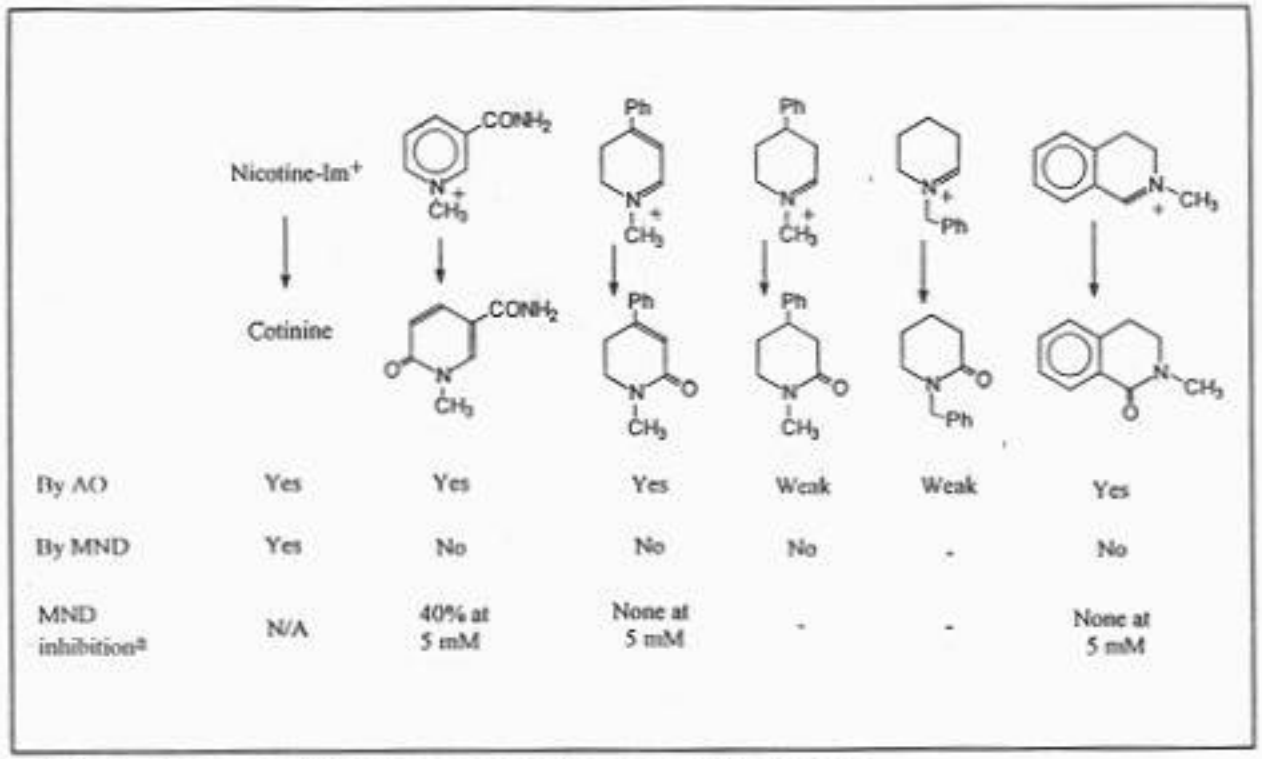


FIGURE 7. *Pathways of phencyclidine metabolism.*

In studies designed to begin characterization of MND (Flammang 1994), it was found that MND activity could be destroyed by delipidation of the microsomes (exposure to detergent), and that activity of the protein pellet could be restored by addition of dilauroyl phosphatidyl choline. Kinetic studies revealed activities associated with both a low- and high-affinity binding of NAD^+ but with a singular binding affinity of the iminium (Flammang 1994). Also, microsomes obtained from



By AO	Yes	Yes	Yes	Weak	Weak	Yes
By MND	Yes	No	No	No	-	No
MND inhibition[a]	N/A	40% at 5 mM	None at 5 mM	-	-	None at 5 mM

TABLE 3. *Conversion of iminium species to the corresponding lactams.*

KEY: a = Inhibition of conversion of nicotine-Im$^+$ to cotinine by microsomes in the presence of NAD$^+$.

phenobarbital-induced rabbits exhibited a less efficient dehydrogenase activity, in contrast to what is observed for aldehyde oxidase-mediated cotinine formation. Clearly, much more work will be needed to elucidate the nature of MND, its endogenous substrates and physiological role, and its substrate-structure profile.

CONCLUSIONS

The most telltale data in terms of mechanism of inactivation of cytochrome P-450 by the methylated 1-BP analogs is provided by the results on ,-DMBP and 4,4-DMBP. In the first case, the fact that the parent amine and iminium intermediate exhibit the same level of enzyme inactivation provides compelling evidence for a metabolic route through the endocyclic enamine. In the case of 4,4-DMBP, one might be tempted to explain the weak inactivation by the parent amine on the basis of a low level of endocyclic iminium formation. However, the independently prepared 4,4-DMBP-Im^+ is itself a weak inactivator. The decreased inactivation seen for 4,4-DMBP-Im^+ can be interpreted in terms of figure 6, wherein 4,4-dimethyl substitution would preclude functionalization at C-4 and would also sterically hinder enzymatic oxygenation at C-3. The fact that 4,4-DMBP-Im^+ still exhibits some inactivation is thus more consistent with C-3 oxygenation being the pathway leading to inactivation. This is also compatible with the decreased inactivation caused by the C-2 methyl group in 2-MBP-Im^+ and 2,6-DMBP-Im^+. Thus, although one cannot deduce at this time the nature of the reactive species generated, cytochrome P-450 oxygenation at C-3 of the enamine appears to be the most reasonable path leading to enzyme inactivation.

The significance of the covalent binding that accompanies the metabolism of nicotine and PCP is uncertain. Although there is presently no direct evidence for acute toxic effects specifically associated with such covalent binding, a role in the toxicologic effects of these or related drugs cannot be excluded at present. Any drug in clinical use has a long list of contra-indications. For any given drug, there is always a percentage of users whose individual biochemical metabolism and/or other drug use creates a situation that causes an adverse response to the new drug. The reasons for this adverse reaction are often not apparent. If drugs with associated covalent binding potential inactivate a sufficiently high percentage of a particular metabolic isozyme, then adverse drug interaction scenarios

may arise. This possibility has been recognized recently in the case of PCP (Owens et al. 1993).
A key focus of this chapter has been on the iminium/enamine stage of metabolism (figure 1) as the critical point governing the balance between toxic activation and detoxication pathways. Thus, although AO and MND efficiently intercept the $^{1.(5.)}$-iminium derived from nicotine, PCP-Im^+ is not a substrate for either enzyme. It seems quite significant that the lack of aldehyde oxidase substrate potential in this case correlates with increased levels of covalent binding of PCP relative to nicotine.

The balance between toxication and detoxication may also vary considerably between different tissues. For example, whereas microsomal rates of conversion of nicotine to the $^{1.(5.)}$-iminium ion for rabbit liver and lung are comparable on a per weight basis, nicotine is oxidized at a higher rate by lung microsomes than by liver microsomes when one corrects for the much smaller P-450 content of lung microsomal protein (McCoy, unpublished data). The higher rate of nicotine metabolism in the rabbit lung results from the enrichment in P-450 isozymes which preferentially metabolize nicotine to the $^{1.(5.)}$-iminium ion, for example, CYP2B4 and CYP4B1, which account for 90 percent of lung P-450 content (Serabjit-Singh et al. 1979) but less than 20 percent of the liver total P-450 content (Lu and West 1980). Further studies (Flammang 1994) demonstrate very low aldehyde oxidase activity in rabbit lung compared to liver, and also very low MND activity in lung compared to liver, as also reported by Obach and Van Vunakis (1990). Thus because of the high nicotine oxidation rate and the poor iminiumøcotinine activity in lung tissue, the potential exists for the intracellular concentration of nicotine $^{1.(5.)}$-iminium to be much greater in lung than in liver. Covalent binding to tissue macro-molecules (Williams et al. 1990) resulting from high intracellular concentrations of the $^{1.(5.)}$-iminium or its metabolites may thus be especially problematic in the lung.

Many naturally occurring and synthetic drugs directed at biogenic amine receptors contain a basic tertiary amine center. It is common practice in the development of new central nervous system (CNS)-active pharma-ceuticals to incorporate pyrrolidino, piperidino, or morpholino moieties (in addition to dimethylamino) in structure-activity investigations. A significant number of cyclic tertiary amine drugs have thus appeared (and continue to appear) in the marketplace despite the fact that this structural construct appears to be especially

prone to generating reactive intermediates during oxidative metabolism.

In some instances, avoiding reactive intermediate generation in the first place may be possible. If one has the choice between two pharmacologically equivalent drug analogs, only one of which has a covalent binding potential, it is clear which analog would be more desirable. The work summarized here was aimed at elucidating any patterns that might permit strategic elimination of metabolism-dependent covalent binding potential. It may be a simple matter of using 4,4-dimethylpiperidino instead of piperidino, for example, as long as the desired biological activity is not compromised. Information leading to prevention of potential toxicity should be quite welcome.

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