N-Oxygenation of Phenethylamine to the trans-Oxime by Adult Human Liver Flavin-Containing Monooxygenase and Retroreduction of Phenethylamine Hydroxylamine by Human Liver Microsomes

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ABSTRACT
The biogenic amine phenethylamine has been shown to be N-oxygenated by human flavin-containing monooxygenase (FMO) (form 3) and human liver microsomes and, to a much lesser extent, N-oxygenated by porcine liver FMO1 and porcine liver microsomes but not by rabbit FMO2. Adult human liver microsomes catalyze the NADPH-dependent N-oxygenation of phenethylamine to the corresponding trans-oxime through the intermediacy of phenethyl hydroxylamine. In addition to trans-oxime formation, phenethyl hydroxylamine is retroreduced to phenethylamine in the presence of human or porcine liver microsomes. Studies on the biochemical mechanism of N-oxygenation suggested that trans-oxime formation was dependent on the human FMO (form 3) and that retroreduction was stimulated by superoxide and dependent on a cytochrome P-450 system. These conclusions are based on studies examining the effects of incubation conditions on phenethylamine N-oxygenation and the effect of reactive oxygen species on phenethyl hydroxylamine retroreduction, respectively. The pharmacological activity of synthetic phenethyl hydroxylamine and phenethyl oxime with a number of biogenic amine receptors and transporters was examined in vitro. In all cases examined, the affinity of phenethyl hydroxylamine and the corresponding oxime for a biogenic amine receptor or transporters was very poor. The results suggest that the biogenic amine phenethylamine is efficiently sequentially N-oxygenated in the presence of human liver microsomes and cDNA-expressed FMO (form 3) to phenethyl hydroxylamine and then to oximes that are pharmacologically inactive and serve to terminate biological activity. N-Oxygenation of phenethylamine to the corresponding trans-oxime is a detoxication process that abrogates pharmacological activity.

Humans are exposed to biogenic amines from a number of food sources, including fish products, cheese, beer and wine, meat and other fermented foods (Stratton et al., 1991). Generally, biogenic amines are formed as the result of ubiquitous normal metabolic activity in animals, plants and microorganisms. Biogenic amines can also be biosynthesized by enzymatic decarboxylation of the corresponding amino acid in bacteria (Brink et al., 1990). In addition, biogenic amines may arise from spoilage of raw materials or bacterial contamination and microbial activity related to fermentation during processing (Izquierdo-Pulido et al., 1993, 1995).

Among number of biogenic amines in food, histamine and tyramine have been implicated in toxicological problems from human ingestion of food and beverages containing biogenic amines (Morrow et al., 1991; Smith, 1981). Other amines, most notably phenethylamine, cadaverine, spermine and spermidine, may function as potentiators that enhance the toxicity of histamine and tyramine (Joosten, 1988; Stratton et al., 1991; Taylor, 1986). The “histamine intoxication” described above is distinct from the well known hypertensive crises brought about in humans treated with MAO inhibitors who have ingested elevated amounts of biogenic amines (the so-called cheese effect) (Lippman and Nash, 1990; Stockley, 1993). In some cases, individuals treated with MAO inhibitors who ingest elevated levels of biogenic amines had a fatal reaction (Stockley, 1993). In the presence of MAO inhibitors, which cause escape from oxidative deamination (and other metabolic processes described herein), ingestion of phenethylamine causes the release of catecholamines present in ele-
vated amounts due to MAO inhibition at nerve endings and the adrenal medulla (Baldessarini, 1982).

The metabolism of phenethylamine has been described in considerable detail. Among the enzymes currently known to metabolize phenethylamine are MAO and CYP. MAO-B preferentially oxidizes noncatecholamines like phenethylamine to the corresponding aldehyde intermediate, which is subsequently hydrolyzed to the aldehyde (Cashman, 1997). This terminates the action of the neurotransmitter. Interestingly, MAO-B activity increases with age (or, alternatively, MAO-A-containing neurons are degenerated) in rat and human brain (Yu, 1994). In humans, studies have linked MAO deficiency with unusual mental behavior (de la Chapelle et al., 1985) or mental retardation (Neri et al., 1992). Alteration in the structural gene was also reflected in abnormal monoamine metabolism (Bleeker-Wagemakers et al., 1988).

The exception of phenethylamine, CYP has also been shown to oxidize substituted 2-phenethylamines to N-hydroxylamines (Lindeke et al., 1985). The 2-phenethyl hydroxylamines are further metabolized to the nitroso compound via the N-hydroxy amino acid by apparently not releasing the substrate from the enzyme surface (Du et al., 1995). The involvement of flavin monoxygenases (Dawson et al., 1993) and peroxidase-like enzymes (Ludwig-Muller et al., 1990) has also been implicated in the conversion of amino acids to oximes in the biosynthesis of glucosinolates.

There have been a few reports stating that the mammalian FMO catalyzes the formation of oximes from aliphatic primary amines (Clement et al., 1993; Lin et al., 1996; Poulsen et al., 1986). Of the five subfamilies of FMO (i.e., FMO1–FMO5), only FMO2 (Poulsen et al., 1986; Tynes et al., 1985; Williams et al., 1984), FMO3 (Cashman, 1995; Lin et al., 1996; Lomri et al., 1993) and FMO5 (Overy et al., 1995) have been reported to oxidize primary amines. Rabbit FMO2 was found to have high activity toward a number of primary amines but low activity toward phenethylamine (Tynes et al., 1986). Primary alkylamines (i.e., n-octylamine) are known stimulators of porcine FMO1 but have not been directly examined as substrates. With the exception of three reports (Clement et al., 1993; Lin et al., 1996; Poulsen et al., 1986), the direct quantification of FMO-mediated N-oxygenation of primary amines has not been reported.

In this report, we describe the in vitro N-oxygenation of phenethylamine and phenethyl hydroxylamine to the corresponding trans-oxime (fig. 1). Phenethylamine is efficiently N-oxygenated in the presence of adult human liver microsomes and highly purified human FMO3. The substrate selectivity was quite pronounced; although porcine FMO1 was competent to N-oxygenate phenethylamine to the trans-oxime, oxime formation was only a fraction of the rate observed for human FMO3. In the presence of adult human liver microsomes, phenethyl hydroxylamine was efficiently converted by retroreduction back to phenethylamine (fig. 1). Studies of the pharmacological activity of phenethyl hydroxylamine and phenetyl oxime suggested that N-oxygenation largely abrogates biological activity on the basis of metabolite interaction with 5-HT and dopamine receptors and the dopamine transporter. Formation of trans-phenethyl oxime by human FMO3 apparently represents a detoxication process and terminates the pharmacological activity of phenethylamine.

Methods

Chemicals

Chemicals used in this study were of the highest purity available from commercial sources. Phenylacetaldehyde, benzyl cyanide, hydroxylamine hydrochloride, sodium cyanoborohydride and phenethylamine were purchased from Aldrich Chemical (Milwaukee, WI). Other buffers, reagents and solvents were obtained from Fisher Chemical (San Jose, CA). All of the compounds of the NADPH-generating system were from Sigma Chemical (St. Louis, MO). Chromatography was done with Silica Wolaem (35–70 mesh; Acros, Pittsburgh, PA).

Instrument Analysis

1H NMR spectra were recorded on a Varian Spectrometer operating at a frequency of 300 MHz. The proton chemical shift values are given in ppm relative to tetramethylsilane. Mass spectra were recorded on a VG 70SEQ instrument. Both instruments are housed at the Department of Medicinal Chemistry, University of Washington, Seattle, WA.

Synthetic Procedures

cis- and trans-Phenethyloximes (3a and 3b).

Phenylethylaldehyde (1.00 g, 8.33 mmol) was dissolved in 15.0 ml of tetrahydrofuran. Hydroxylamine hydrochloride (695 mg, 10.0 mmol) was dissolved in 4.0 ml of 1.0 mg/ml solution) as an indicator to prevent over reduction. Sodium cyanoborohydride (1.0 M solution in methanol) (74 mg, 0.74 mmol) were dissolved in 5.0 ml of MeOH with bromophenol blue (74 µl of 1.0 mg/ml solution) as an indicator to prevent over reduction. Sodium cyanoborohydride (1.0 M solution in tetrahydrofuran, 490 µl, 0.67 Eq) was added to the reaction mixture,
and 

and HCl (10%) was added dropwise as needed to maintain a yellow color until the yellow color was persistent. The resulting mixture was stirred an additional 30 min at room temperature, and the thin-layer chromatography (silica, 0.2% TEA, 5% MeOH in CH₂Cl₂) indicated the reaction was complete. The reaction mixture was treated with 10% Na₂CO₃ and extracted twice with ethyl acetate. The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated to give a solid in vacuo. The pure product 2 was obtained after flash chromatography (silica, 0.2% TEA, 5% MeOH in CH₂Cl₂) to yield a solid. Yield was 40%; 1H NMR (in CDCl₃): 8 3.45 (t, J = 7.7 Hz, 2H), 3.55 (m, 2H), 7.26 (m, 5H); MS (FAB): 138 (MH⁺), 120 (MH⁻ – OH), 105 (MH⁻ – OHN).

Liver and Microsome Preparations

The adult human liver microsome samples were obtained under a protocol approved by the Committee for the Conduct of Human Research at the Medical College of Wisconsin. Adult human liver microsomes were a generous gift of Dr. Steven A. Wrighton (Eli Lilly and Company, Indianapolis, IN). The CYP content and specific activity of selected CYP were determined as previously described (Lowry et al., 1951; Omura and Sato, 1964). Each of the eight most prominent CYPs has been completely characterized immunchemically as well as by selective functional substrate assays: ethoxyresorufin-O-deethylase, coumarin-7-hydroxylase, S-mephenytoin-4'-hydroxylation, bufuralol-1'-hydroxylase, N-nitroso-dimethylamine N-demethylase and erythromycin N-demethylase (25, 440, 103, 43, 661 and 249 pmol of product/mg of microsomal protein/min, respectively) (Wrighton et al., 1993). The CYP content was 317 pmol of CYP/mg of protein. The FM03 has likewise been previously fully characterized from these preparations (Cashman et al., 1992; Wrighton et al., 1993), and the nicotine N-1'-oxygenase activity was 125 pmol/min/mg of protein.

Metabolic Incubation Systems

A typical incubation mixture contained 50 mM potassium phosphate buffer, pH 9.0, 0.4 mM NAD⁺, 0.4 mM glucose-6-phosphate, 1.0 IU of glucose-6-phosphate dehydrogenase, 40 to 100 μg of cDNA-expressed human liver FM03-MBP2 or 0.40 to 0.60 mg of adult human liver microsomes and 1.2 mM DETAPAC (final volume, 0.25 ml). The reaction was initiated by the addition of substrate and incubated at 37°C with shaking in air. At various time intervals, the incubations were stopped by the addition of 1.5 ml of cold dichloromethane. After saturation with Na₂CO₃ and a brief centrifugation, the organic layer was separated from the aqueous phase, evaporated and then analyzed for products by the HPLC procedure described below.

The profile of phenethylamine metabolites was determined by HPLC analysis of dichloromethane extracts of the incubation mixture. The metabolic products were separated and quantified with a Hitachi L-6200A HPLC interfaced to a Hitachi D-2500 Chromato-Integrator with a Hitachi L-4000UV UV detector set at 257 nm. The system was fitted with a C-18 analytical column (25 × 0.4 cm) from Rainin (Emeryville, CA). The mobile phase consisted of an isotropic system set at 70% of A and 30% of B, in which A is water containing 1.0% of THF and B is CH₃CN containing 1.0% of HClO₄ (60% solution) at a flow rate of 1.5 ml/min. This system efficiently separated phenethylamine, phenethyl hydroxylamine and trans- and cis-oximes that had retention times of 3.02, 3.43, 5.93 and 7.05 min, respectively. On the basis of UV absorption of each material, metabolites were quantified by comparing the metabolite and substrate peak areas of the chromatogram and calculated according to the following formula: % conversionoximes = areas of oximes/areas of oximes + 2.0 areas of phenethylamine), or % conversionoximes = areas of oximes/(areas of oximes + 4/3 areas of phenethyl hydroxylamine).

Immunoblotting and Antisera

Antibodies that recognized specific human CYP were used to characterize the microsome CYP immunoreactivity as previously described (Wrighton et al., 1993). Antibodies to guinea pig FM03 were raised in rabbits and used to detect human FM03 by a method similar to one previously described (Guan et al., 1991). The antibody to guinea pig FM03 was a generous gift of Drs. K. Oguri and H. Yamada (Kyushu University, Fukuoda, Japan).

Receptor Binding Assays

HA7 cells were grown to confluence in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 0.05% penicillin-streptomycin and 400 μg/ml G418. The cells were scraped from 100 × 20-mm plates and centrifuged at 500 × g for 5 min. The pellet was homogenized at 2 plates/ml in 50 mM Tris-HCl, pH 7.7, with a Polytron as previously described (Sunahara et al., 1991). For 5-HT_1A receptor, the competitive assay was done with [³H]8-hydroxy-2-(diisopropylamino)tetralin. The tubes were incubated at 25°C for 60 min and then filtered through a Whatman GF/B filter paper on a Brandel cell harvester, and the filters were counted by scintillation counting.

For the 5-HT_1C receptor, NIH-3T3-Pc cells were grown and prepared in the same manner as HA7 cells. [³H]Mesulergine was used to measure competitive binding. For the 5-HT_3A receptor, NIH-3T3-GF6 cells were grown as described above, and [³H]ketanserin was used to measure competitive binding. For the 5-HT_3B receptor, NG108–15 cells were grown as above, and [³H]GR65630 was used to measure competitive binding. For the D₁ receptor, LHD1 cells were grown as described above, and competitive binding was determined with [³H]SCH 13,390. For D₂ and D₃ receptors, CHOP-D₃ and CHOP-D₃ cells, respectively, were grown as described above, and [³H]YM-09151–2 was used in competitive binding experiments. For dopamine transporter binding studies, C6 hDAT cell membranes are prepared as previously described (Eshleman et al., 1995), and competitive binding was done with [³H]RTI-55.

Results

Chemical synthesis and stability. The chemical synthesis of metabolites of phenethylamine was done to obtain sufficient material for the identification of phenethylamine metabolites in vitro and to study the chemical stability of these materials under the aqueous incubation and analysis conditions. In addition, because the N-oxidative metabolites of phenethylamine are virtually completely uncharacterized from a pharmacological point of view, sufficient material was synthesized for in vitro pharmacological evaluation with selected receptors and transporters.

Treatment of phenylacetaldehyde with hydroxylamine hydrochloride under basic conditions produced a mixture of cis- and trans-oximes, 3 (fig. 2) (Lin et al., 1996). The oximes were reduced to the desired hydroxylamine 2 in the presence of NaCNBH₃ under acidic conditions. The reduction conditions used were acidic (pH < 2); otherwise, the dimer [Ph(CHOH)₃N(OH)(CHOH)₃Ph, MS (FAB): 242 (MH⁺)] was formed instead of the desired hydroxylamine 2. Each purified compound was completely characterized by spectral means, and an HPLC system was developed to separate the oximes and hydroxylamine of phenethylamine.

As a prelude to the metabolic studies, the chemical stability of primary amine 1, hydroxylamine 2 and oximes 3a and 3b to hydrolysis and autoxidation was determined to identify degradation products and define any possible restriction on
the use of the amine or metabolites during the metabolic incubations or during the analysis. In addition, the oximes were examined for their ability to isomerize during the experimental conditions used. The primary amine 1 and the hydroxylamine 2 were found to be completely stable under the reaction conditions that were used. Thus, incubation of 1 or 2 in potassium phosphate buffer (pH 8.0 or 9.0), extraction into dichloromethane, evaporation and reconstitution into acetonitrile and analysis by HPLC showed no detectable decomposition of either compound. In contrast, the oximes, compounds 3a and 3b, although stable to hydrolysis and autoxidation, did undergo very modest cis-to-trans-isomerization under the conditions of the experiment and analysis. Incubation of a previously defined amount of cis-trans-isomerization (i.e., 8:1 ratio as determined by 1H NMR and HPLC) at pH 8.0 or 9.0 generally showed a detectable isomerization to the trans-oxime. However, cis-trans-isomerization of oxime 3 was not completely reproducible and may have been dependent on minute amounts of water present in the extraction solvent (i.e., dichloromethane) or minute amounts of water present in the reconstitution solvent (i.e., acetonitrile) used in the HPLC analysis. Aqueous incubation of oxime 3 worked up in the normal fashion and analyzed by UV-vis or used in the HPLC analysis. Aqueous incubation of oxime 3b, dichloromethane) or minute amounts of oxime 3a, although stable to hydrolysis and autoxidation, did not undergo any detectable isomerization to the trans-oxime. However, cis-trans-isomerization of oxime 3 was not completely reproducible and may have been dependent on minute amounts of water present in the extraction solvent (i.e., dichloromethane) or minute amounts of water present in the reconstitution solvent (i.e., acetonitrile) used in the HPLC analysis. Aqueous incubation of oxime 3 worked up in the normal fashion and analyzed by UV-vis or high field 1H NMR spectroscopy for other by-products did not show any other detectable products (e.g., dimer). The results suggest that the phenethyl oximes 3a and 3b were stable to hydrolysis and autoxidation during the conditions of metabolic incubations and analysis used but that the determination of stereoselectivity observed probably slightly overreported the amount of trans-oxime formed. However, because we could not detect significant quantities of the cis-oxime 3a formed during the studies, the results suggest that very high stereoselectivity was observed.

**Metabolism of phenethylamine and phenethylamine hydroxylamine in the presence of microsome preparations.** The metabolism of phenethylamine in the presence of various microsome preparations was done to determine the nature of the monooxygenases responsible for the transformation and identify the products formed. Porcine liver microsomes provided a convenient source of porcine FMO1, and adult human liver microsomes provided a source of human FMO3. Both preparations possessed good levels of highly characterized CYP activity (Decker et al., 1992; Lin et al., 1996). In the presence of porcine liver microsomes or adult human liver microsomes, aerobic incubation of phenethylamine resulted in exclusive formation of trans-oxime 3b (i.e., 0.22 ± 0.04 and 3.74 ± 0.56 nmol/min/mg of protein, respectively). In the presence of porcine liver microsomes, formation of oxime 3b from phenethylamine was linearly dependent on time (i.e., 0–15 min) and protein concentration (i.e., 0–1.7 mg of protein). Likewise, in the presence of adult human liver microsomes, formation of oxime 3b was linearly dependent on time (i.e., 0–10 min) and protein concentration (i.e., 0–0.76 mg of protein). Under the experimental conditions used, formation of phenethylamine hydroxylamine was not observed in the presence of either porcine or human liver microsomes. The results were consistent with figure 1. Formation of oxime 3b was completely dependent on NADPH and active protein. It was notable that adult human liver microsomes were almost 17-fold more efficient than porcine liver microsomes in forming oxime 3b from phenethylamine despite the fact that porcine liver microsomes are generally 50- to 50-fold more active than adult human liver microsomes with regard to tertiary amine N-oxidation.

**Effect of incubation conditions on oxime formation from phenethylamine and phenethyl hydroxylamine**

To identify the hepatic monooxygenase(s) predominantly responsible for phenethylamine metabolism, we used different metabolic incubation conditions. Human liver microsomes were used because considerably more product was formed. As described above, in the presence of porcine and adult human liver microsomes, formation of oxime 3b from phenethylamine was dependent on NADPH and active microsomal protein. Two well-documented alternative substrate competitive inhibitors of FMO (i.e., thiourea and thio- benzamide) significantly decreased the formation of oxime 3b in the presence of human liver microsomes. n-Octylamine, a compound that inhibits CYP (Jefcoate et al., 1969) and decreases human FMO3 activity (Cashman, 1995; Lomri et al., 1993), depressed the amount of oxime 3b formed in the presence of human liver microsomes (table 1). Under conditions of heat inactivation that generally abolished adult human liver microsomal FMO activity and left CYP activity intact (Cashman et al., 1993), oxime 3b formation was drastically decreased but not completely abrogated (see table 1).

Because we anticipated that phenethyl hydroxylamine was an obligatory intermediate in the transformation of phenethylamine to its corresponding oxime metabolite and we did not observe hydroxylamine 2 formation, we investigated the microsomal metabolism of phenethyl hydroxylamine. The hypothesis that was tested was that oxime 3b formation constituted an A→B→C reaction (fig. 1) and that the second step (i.e., B→C) was faster than the first and therefore the accumulation of hydroxylamine 2 was negligible. This hypothesis also predicts that hydroxylamine 2 does not become

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**TABLE 1**

<table>
<thead>
<tr>
<th>N-Oxygenation of phenethylamine 1 by human liver microsomes</th>
<th>trans-Oxime&lt;sup&gt;a&lt;/sup&gt; (nmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.67 ± 0.66</td>
</tr>
<tr>
<td>+N-Octylamine (5.0 mM)</td>
<td>2.58 ± 0.90 (29.7%)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>+Thiourea (5.0 mM)</td>
<td>2.03 ± 0.17 (44.7%)</td>
</tr>
<tr>
<td>+Thiobenzamide (5.0 mM)</td>
<td>1.80 ± 0.18 (51.0%)</td>
</tr>
<tr>
<td>+Heat inactivation (+10 units catalase)</td>
<td>0.94 ± 0.12 (74.4%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The complete system contained 1.2 mM phenethylamine 1, 0.4 mM NADPH, 0.4 mM glucose-6-phosphate, 1 IU of glucose-6-phosphate dehydrogenase, 1.2 mM DETAPAC and 0.57 mg of adult human liver microsomes in potassium phosphate buffer, pH 8.0. The incubation time was 10 min at 37°C.

<sup>b</sup> Data are expressed as mean ± S.D. of 3 determinations. The limit of detection was 50 pmol of oxime/min/mg of protein.

<sup>c</sup> Percent inhibition is provided in parentheses.
a metabolite that freely dissociates from the enzyme surface. In the presence of porcine or adult human liver microsomes, two products were observed to be formed from hydroxylamine 2: phenethylamine 1 was formed in an apparent retroreductive step (i.e., 0.83 ± 0.06 and 1.7 ± 0.33 nmol/min/mg of protein, respectively), and the trans-oxime 3b was formed from an apparent oxidative pathway (i.e., 3.41 ± 0.55 and 5.6 ± 0.78 nmol/min/mg of protein, respectively). In the presence of porcine liver microsomes, formation of oxime 3b from hydroxylamine 2 was linearly dependent on time (i.e., 0–15 min) and protein concentration (i.e., 0–1.55 mg of protein). In the same microsome preparation, retroreduction of phenethylamine hydroxylamine to the corresponding primary amine was linearly dependent on time (i.e., 0–15 min) and protein concentration (i.e., 0–0.90 mg of protein). Likewise, in the presence of adult human liver microsomes, formation of oxime 3b and primary amine 1 from hydroxylamine 2 were linearly dependent on time (i.e., 0–8.0 min and 0–10 min, respectively) and of protein concentration (i.e., 0–0.76 mg of protein).

In the presence of porcine liver microsomes and adult human liver microsomes, formation of oxime 3b and phenethylamine 1 from phenethylamine hydroxylamine was completely dependent on NADPH and active protein. Because oxime 3b formation from hydroxylamine 2 was only 1.6-fold more efficient in the presence of human liver microsomes than porcine liver microsomes, the results suggested that hydroxylamine 2 was a much better substrate for porcine FMO1 than the primary amine, 1.

Effects of inhibitors on oxime formation from phenethyl hydroxylamine in porcine and human liver microsomes. Formation of trans-oxime 3b from phenethyl hydroxylamine 2 was highly dependent on NADPH and active protein. In the presence of porcine liver microsomes, the alternate substrate competitive inhibitor thiourea decreased oxime 3b formation but not as significantly as that observed when phenethylamine was incubated with human liver microsomes (table 2). The addition of n-ocytalamine, a compound that inhibits CYP and stimulates porcine FMO1 activity, increased trans-oxime 3b formation (table 2). In the presence of human liver microsomes, the alternate substrate competitive inhibitors thiourea and thiobenzamide decreased oxime 3b formation (table 3). The addition of n-ocytalamine did not have a marked effect on the formation of trans-oxime 3b from phenethyl hydroxylamine (table 3), and heat inactivation of both microsome preparations significantly decreased trans-oxime 3b formation (tables 2 and 3). The results are consistent with the involvement of a CYP in the retroreduction of hydroxylamine 2 to primary amine 1.

Role of highly purified FMOs in phenethylamine N-oxygenation. To examine a role of FMO in phenethylamine oxime 3b formation, the relative rates of oxime formation in the presence of highly purified preparations of porcine FMO1, rabbit FMO2 and cDNA-expressed human Lys158 FMO3 (3) and Lys3-maltese binding protein (Lys3 MF03-MBP) were determined. Preliminary studies indicated that porcine FMO1 and rabbit FMO2 showed modest and negligible rates of oxime 3b formation, respectively (table 4). This was not due to lack of FMO activity because separate studies showed that porcine FMO1, rabbit FMO2 and human Lys158 FMO3-MBP possessed high activity as a tertiary amine N-oxygenase using the substrate 10-[(N,N-dimethylnopentyl)-2-(trifluoromethyl)phenothiazine. The results with rabbit FMO2 are in agreement with previously established structure-activity relationships (Nagata et al., 1990) that suggest that long aliphatic amine substrates are required for enzyme activity.

Although this is the first report showing extremely modest primary amine N-oxygenase activity for porcine FMO1, cDNA-expressed human Lys158 FMO3-MBP showed quite high activity for oxime 3b formation with phenethylamine and was investigated in considerable detail. Preliminary studies with cDNA-expressed human Lys158 FMO3-MBP showed that tertiary amine N-oxygenase activity was opti-

### TABLE 2

**N-Oxygenation of phenethylamine hydroxylamine 2 by porcine liver microsomes**

<table>
<thead>
<tr>
<th>Description</th>
<th>Product&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RNH₂</th>
<th>trans-Oxime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+n-Octylamine (5 mM)</td>
<td>0.75 ± 0.10</td>
<td>2.71 ± 0.06</td>
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</tr>
<tr>
<td>Thiourea (5 mM)</td>
<td>0.48 ± 0.13</td>
<td>3.02 ± 0.68</td>
<td></td>
</tr>
<tr>
<td>Thiobenzamide (5 mM)</td>
<td>0.72 ± 0.05</td>
<td>2.07 ± 0.11</td>
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</tr>
<tr>
<td>Heat inactivation (10 units catalase)</td>
<td>0.76 ± 0.18</td>
<td>N.D.&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The complete system was as described in table 1 except it contained 1.2 mM phenethylamine hydroxylamine 2 and 0.82 mg of porcine liver microsomes.
<sup>b</sup> Data are expressed as mean ± S.D. of 3 determinations.
<sup>c</sup> N.D., nondetectable. Limit of detection was 50 pmol/min/mg of protein.

### TABLE 3

**N-Oxygenation and retroreduction of phenethylamine hydroxylamine 2 by human liver microsomes**

<table>
<thead>
<tr>
<th>Description</th>
<th>Product&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RNH₂</th>
<th>trans-Oxime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+n-Octylamine (5 mM)</td>
<td>1.86 ± 0.40</td>
<td>5.83 ± 0.65</td>
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<tr>
<td>Thiourea (5 mM)</td>
<td>0.36 ± 0.05</td>
<td>5.24 ± 0.81</td>
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<tr>
<td>Thiobenzamide (5 mM)</td>
<td>1.30 ± 0.01</td>
<td>5.33 ± 0.69</td>
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<tr>
<td>Heat inactivation (10 units catalase)</td>
<td>1.18 ± 0.13</td>
<td>4.49 ± 0.40</td>
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</tbody>
</table>

<sup>a</sup> The complete system was as described in table 1 except it contained 1.2 mM phenethylamine hydroxylamine 2 and 0.57 mg of adult human liver microsomes.
<sup>b</sup> Data are expressed as mean ± S.D. of 3 determinations.

3 Restriction length polymorphism and oligonucleotide-sequencing studies showed that codon 158 encoded either amino acids Glu or Lys at approximately equal allele frequencies for the Caucasian populations examined (E. Treacy, R. Youil, S. Forest and M. Knight, unpublished observations).
In the presence of porcine FMO1, rabbit FMO2 and cDNA-expressed human Lys<sup>158</sup> FMO3-MBP, hydroxylamine 2 appeared to be a better substrate for formation of oxime 3b than the phenethylamine 1 (table 4). Formation of oxime 3b from phenethyl hydroxylamine 2 was linearly dependent on time (i.e., 0–12 min) and protein concentration (i.e., 0–80 μg of protein). Formation of oxime 3b was examined with a variety of metabolic incubation conditions (table 5). Phenethyl oxime 3b formation from hydroxylamine 2 was highly dependent on active human Lys<sup>158</sup> FMO3-MBP protein and NADPH. The alternate substrate competitive inhibitor thiobenzamide decreased oxime 3b formation by >40%. n-Octylamine did not affect the formation of oxime 3b from phenethyl hydroxylamine 2, which suggested that long-chain aliphatic primary amines were poorer substrates for Lys<sup>158</sup> FMO3-MBP than phenethylamine. Heat inactivation of human Lys<sup>158</sup> FMO3-MBP in the absence of NADPH completely abolished oxime 3b formation activity. In the presence of human Lys<sup>158</sup> FMO3-MBP, oxime 3b formation from phenethyl hydroxylamine paralleled the results observed for oxime formation from phenethyl hydroxylamine in the presence of microsomes. The data suggested that hydroxylamine 2 was a transient intermediate in the formation of oxime 3b in an apparent A→B→C reaction (fig. 1), where the B→C component is much faster than the A→B step. Under the experimental conditions used, in the presence of cDNA-expressed human Lys<sup>158</sup> FMO3-MBP, no detectable retroreduction of hydroxylamine 2 to primary amine 1 was observed. In agreement with the microsome studies described above, once the hydroxylamine 2 was formed, no evidence for dissociation from the surface of FMO was apparent.

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ence or absence of exogenously added H$_2$O$_2$, no significant alteration of phenethylamine formation was observed. However, generation of superoxide markedly increased retroreduction of phenethylamine hydroxylamine, whereas abrogation of superoxide by the action of SOD completely abolished retroreduction of hydroxylamine 2 (table 6).

Finally, to investigate a role of reactive oxygen species in the human Lys$^{158}$ FMO3-MBP-catalyzed oxime 3b formation, as shown in table 7, human Lys$^{158}$ FMO3-MBP-catalyzed oxime 3b formation was essentially independent of the presence of H$_2$O$_2$ and/or O$_2$. In the presence of human Lys$^{158}$ FMO3-MBP, reactive oxygen species had no effect on retroreduction of hydroxylamine 2, and no detectable amount of primary amine 1 was observed.

**Kinetic determination of trans-oxime 3b formation by prominent human FMO3s.** The substrate dependence for the N-oxygenation of phenethylamine and phenethylamine hydroxylamine was examined in the presence of cDNA-expressed human Lys$^{158}$ and Glu$^{158}$ FMO3s (table 8). The two human FMO3 enzymes were studied because they represent the most prominent FMO3 enzymes present in normal adult humans. Thus, plots of the reciprocal of velocity vs. the reciprocal of the substrate concentration provided a series of linear correlations. From these Lineweaver-Burk plots, the $K_{mapp}$ and $V_{max}$ values were obtained (table 8). For both phenethylamine and phenethylamine hydroxylamine, it appeared that the $K_{mapp}$ value for Lys$^{158}$ FMO3 was elevated compared with that of the human Glu$^{158}$ FMO3-MBP enzyme.

**Effect of hydroxylamine 2 or oxime 3 on biogenic amine transporter and receptors.** The binding affinity for phenethylamine hydroxylamine 2 and oxime 3 for several biogenic amine receptors and the dopamine transporter were examined. Thus, the 5-HT receptors (i.e., human 5-HT$\text{1C}$, rat 5-HT$\text{1C}$, rat 5-HT$\text{2A}$, and guinea pig 5-HT$\text{3b}$), the dopamine receptors (i.e., human D$_{1}$, D$_{2}$ and D$_{3}$) and the dopamine transporter (i.e., human DAT) were examined for competitive binding to well established ligands. As shown in table 9, the IC$_{50}$ or $K_{i}$ determinations all showed very poor avidity with values of >10 $\mu$M. Such IC$_{50}$ or $K_{i}$ values are beyond the physiological range and suggest that hydroxylamine 2 and oxime 3 do not possess any significant pharmacological activity for these receptors.

**Discussion**

An efficient method was developed to synthesize phenethylamine hydroxylamine 2 and phenethyl oxime 3. The cis- and trans-oximes were separated by fractional recrystallization; this allowed sufficient material to conduct biochemical and pharmacological studies. High-resolution proton NMR studies confirmed the identity of the oxime stereoisomers. In contrast to many previous studies that have shown that cis-oximes are preferentially formed metabolically, only the trans-oxime 3b was observed to be formed. The oxime 3 had a slight tendency to isomerize to the trans-oxime 3b; however, this did not interfere with the determination of the stereochemistry of the enzymatic product. Using ion-pair chromatography, a convenient HPLC procedure was developed to separate and quantify phenethylamine 1 and the corresponding hydroxylamine 2 and cis- and trans-oximes 3 (fig. 1).

Metabolic studies suggested that the only product observed to be formed from phenethylamine 1 in the presence of porcine or adult human liver microsomes was the trans-oxime 3b. No evidence for the formation of hydroxylamine 2 from phenethylamine was observed. It is possible that catalytic facilitation (Du et al., 1995) channels the flow of phenethylamine metabolites through a series of intermediates that are tightly bound to FMO. This may account for the failure to observe dissociable hydroxylamine 2 along an A→B→C reaction path leading to exclusive formation of trans-oxime 3b.

Previous studies using rat liver microsomes from phenobarbital-treated animals showed that phenethylamine was a poor substrate for N-hydroxylation (Jonsson and Lindke, 1976). Unlike substituted 2-phenethylamines, phenethylamine does not form metabolic inhibitory complexes, and thus, the lack of N-oxygenation in a particular microsome preparation for phenethylamine is not due to CYP inhibition. More likely, the extent of phenethylamine N-oxygenation is due to the type and amount of FMO present. The relatively low amount of FMO3 and abundance of FMO1 in rat liver preparations (Cashman, 1995) and the difficulty in procuring microsomal FMO activity may account for the prior observation of low N-oxygenation capacity of phenethylamine in rat liver microsomes (Lindke et al., 1982). On the other hand, FMO1 is the only FMO present in porcine liver microsomes, and the data of table 1 show that low, albeit detectable, levels of oxime 3b were formed. Primary amine N-oxygenation by

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**TABLE 6**

<table>
<thead>
<tr>
<th>Description</th>
<th>Product$^b$</th>
<th>RNH$_2$</th>
<th>trans-Oxime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete$^a$</td>
<td>0.19 ± 0.02</td>
<td>0.94 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>+H$_2$O$_2$ (10 mM)</td>
<td>0.16 ± 0.02</td>
<td>1.13 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>+Catalase (100 IU)</td>
<td>0.23 ± 0.05</td>
<td>1.20 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>+H$_2$O$_2$ + catalase</td>
<td>0.19 ± 0.11</td>
<td>1.19 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>+Xanthine/xanthine oxidase (0.25 mM)</td>
<td>0.46 ± 0.01</td>
<td>1.37 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>+SOD (100 IU)</td>
<td>0.19 ± 0.02</td>
<td>1.12 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>+Xanthine/xanthine oxidase + SOD</td>
<td>N.D.$^c$</td>
<td>1.30 ± 0.33</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The complete system was the same as table 1 except it contained 1.2 mM phenethylamine hydroxylamine 2 and 0.57 mg of human Lys$^{158}$ FMO3-MBP in potassium phosphate buffer, pH 9.0.

$^b$ Data are expressed as mean ± S.D. of 3 determinations.

$^c$ N.D., nondetectable. The limit of detection was 50 pmol/min/mg of protein.
FM01 has not been previously reported. Generally, primary aliphatic amines stimulate FM01-mediated substrate oxygenation, although in some cases, inhibition has been observed (Cashman, 1995). Thus, depending on the substrate, an aliphatic primary amine may show alternate substrate competitive inhibition or, alternatively, FM0 stimulatory activity. This may be a consequence of generation of reactive oxygen species. Regardless, the effect is modest compared with FM03-catalyzed N-oxygenation of phenethylamine.

In contrast to porcine FM01, human FM03 efficiently N-oxygenates phenethylamine to produce exclusively trans-oxime 3b. The effect of metabolic inhibitors on phenethylamine N-oxygenation (table 1) is consistent with a prominent role of human FM03 in the formation from phenethylamine, but it does not rule out the involvement of CYP. For example, under heat inactivation conditions that abolish ~85% of FM0 activity, almost 25% oxime formation is still observed (table 1). We conclude that if CYP contributes to human microsome-catalyzed phenethylamine N-oxidation, it is doing so on the order of ~10%.

Although we do not detect phenethylamine hydroxylamine 2 as an observable metabolite under analytical conditions that would detect very low levels (i.e., 50 pmol/min/mg of protein), it is undoubtedly the initial metabolite in the formation of oxime 3b. Incubation of synthetic phenethylamine hydroxylamine 2 in the presence of porcine and human liver microsomes efficiently produced exclusively trans-oxime 3b, confirming that hydroxylamine 2 was indeed on the sequential multistep reaction pathway leading to oxime 3b. The effect of metabolic inhibitors on conversion of hydroxylamine 2 to oxime 3b was consistent with a prominent role of FM0. In the case of porcine liver microsomes, n-oxylamine modestly stimulated oxime 3b formation, whereas in the presence of adult human microsomes, n-oxylamine slightly inhibited oxime 3b formation (table 2 and 3). This shows that n-oxylamine can have a stimulatory or inhibitory effect on FM01 and FM03, respectively, for the N-oxygenation of the same substrate for both enzymes. In the presence of heat inactivation of adult human liver microsomes, phenethylamine hydroxylamine N-oxidation produced ~24% more oxime 3b than expected. It is possible that heat-inactivated adult human liver microsome CYP contributes as much as 24% to the overall oxime 3b formation. This value is somewhat surprising in view of the potent metabolic intermediate complex that 2 can form with CYP (Jonsson and Lindeke, 1976; Lindeke et al., 1982; Mansuy et al., 1978).

Depending on the substrate structure, four of the five known mammalian FM0s are now known to N-oxygenate primary aliphatic amines [i.e., FM01 (this work), FM02 (Poulsen et al., 1986; Tynes et al., 1985; Williams et al., 1984), FM03 (Cashman, 1995; Lin et al., 1996; Lomri et al., 1993) and FM05 (Overby et al., 1995)]. The data of table 4 show that considerable substrate selectivity was observed in comparison of FM01, FM02 and FM03 in the N-oxygenation of phenethylamine 1 and phenethyl hydroxylamine 2. Thus, highly purified porcine FM01 and cDNA-expressed human FM03-MBP catalyzed N-oxygenation of phenethylamine with very modest and very robust efficacy, respectively. Highly purified rabbit FM02 did not detectably N-oxygenate phenethylamine. This is in keeping with previously established structure-activity relations that showed that only long-chain aliphatic amines (i.e., ~5 methylene units separating the aryl group from the primary amine functionality) were required for rabbit FM02 substrate activity (Nagata et al., 1990). Previously, the human FM03 substrate binding site was also thought to be restricted to long-chain amines (Cashman, 1995; Lomri et al., 1993), but apparently, simple phenylalkylamines also possess excellent substrate specificity. In comparison, a previous study with rabbit FM02 showed that aliphatic primary amines were converted predominantly to the cis-oxime at low substrate concentrations but that N-hydroxylamines were formed at high amine concentrations (Poulsen et al., 1986).

Phenethyl hydroxylamine 2 was converted into oxime 3b by all three FM0s examined (table 4). This probably is a consequence of the enhanced nucleophilicity (i.e., α effect) of the hydroxylamine moiety. Porcine liver FM01 and rabbit FM02 catalyzed a modest amount of oxime 3b formation. cDNA-expressed human FM03-MBP was by far the most efficient phenethylamine hydroxylamine N-oxygenase cata-

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### Table 8

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme</th>
<th>$K_{\text{micb}}$</th>
<th>$V_{\text{mic}}$</th>
<th>$V_{\text{mic}}/K_{\text{micb}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenethylamine</td>
<td>Lys$^{105}$ FM03-MBP</td>
<td>0.197</td>
<td>19.3</td>
<td>97.99</td>
</tr>
<tr>
<td>Glu$^{106}$ FM03-MBP</td>
<td>0.0904</td>
<td>28.2</td>
<td>311.9</td>
<td></td>
</tr>
<tr>
<td>Phenethylamine hydroxylamine</td>
<td>Lys$^{105}$ FM03-MBP</td>
<td>0.467</td>
<td>29.9</td>
<td>64.0</td>
</tr>
<tr>
<td>Glu$^{106}$ FM03-MBP</td>
<td>0.083</td>
<td>12.6</td>
<td>200.0</td>
<td></td>
</tr>
</tbody>
</table>

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*The kinetic constants were determined using the incubation system described in table 1 except it contained variable amounts of phenethylamine 1 or phenethylamine hydroxylamine 2 and 99 μg of human Lys$^{105}$ FM03-MBP or Glu$^{106}$ FM03-MBP in potassium phosphate buffer, pH 9.0.*

### Table 9

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT$_{a,b}$</th>
<th>5-HT$_{c,b}$</th>
<th>5-HT$_{m,b}$</th>
<th>5-HT$_{e,b}$</th>
<th>5-HT$_{g,b}$</th>
<th>5-HT$_{d,b}$</th>
<th>5-HT$_{e,b}$</th>
<th>5-HT$_{g,b}$</th>
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<tr>
<td>2</td>
<td>&gt;10</td>
<td>&gt;10</td>
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<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
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<tr>
<td>3</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

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*Values represent the means from at least 2 independent experiments, each conducted with 2 or 3 determinations.

$IC_{50}$ values for the receptors were also determined and were all >10 μM.

Value reported for $K_i$ determination with the transporter. The $K_i$ value for cocaine displacement of [125I]RTI-55 binding was 229 nM.
lyst examined. This observation encouraged a more detailed examination of the mechanism of human FMO3-mediated oxime 3b formation. Affinity chromatography-purified human FMO3-MBP was used to examine the N-oxygenation of 1 and 2. cDNA-expressed human FMO3-MBP offers many advantages to the study of lipophilic amines, including (1) the water-soluble and essentially detergent-free nature of the enzyme, (2) the similarity of the substrate specificity of the nonfusion protein and (3) the enhanced stability of human FMO3-MBP with respect to microsomal FMO3 in the presence of NADPH.

In good agreement with human liver microsome studies, formation of trans-oxime 3b from phenethylamine or phenethylamine hydroxylamine was observed in the presence of cDNA-expressed human FMO3-MBP. Taken together, the data support a prominent role of human FMO3 in the sequential multistep reaction that converts primary amine 1 into oxime 3b (fig. 3). Currently, a mechanism involving N,N-dioxygenation of 2 is favored. No evidence for formation of the CYP-mediated nitroso compound (i.e., intermediate 5) was observed. If formed, on the basis of previous studies, the nitroso compound 5 would be expected to potently inhibit CYP (Jonsson and Lindeke, 1976; Lindeke et al., 1982; Mansuy et al., 1978). It is possible that the nitroso compound 5 could equilibrate with oxime 3, but this would be anticipated to form significant amounts of cis-oxime 3a. That no detectable cis-oxime 3a was observed to be formed in the presence of the microsomes and FMO enzymes that were used suggests that path II (fig. 3) contributed <5% of the overall conversion of phenethylamine 1 to oxime 3b. It is notable that only the trans-oxime 3b is formed from phenethylamine despite the fact that the proposed symmetrical N,N-dihydroxy intermediate would be predicted to provide both oxime stereoisomers (path I, fig. 3). This observation is in keeping with the postulate that the surface of FMO facilitates catalysis and that stereoselective formation of only the trans-oxime 3b is observed despite the likelihood that dehydration is a spontaneous reaction. Although dehydration of the proposed N,N-dihydroxy intermediate is presumably nonenzymatic, other studies of FMO3-mediated NADPH-independent isomerization of other cis-oximes to trans-oximes suggest that FMO3 may provide a protein template for dehydration.

In the presence of porcine or human liver microsomes, retroreduction of hydroxylamine 2 to form the primary amine 1 competed with N-oxygenation to produce oxime 3b. Previously, we have shown that hydroxylamine retroreduction in the presence of human liver microsomes did not correlate significantly with human FMO3 or with any well characterized CYP activity or immunoreactivity (Lin et al., 1996). In contrast to a previous study using rabbit FMO2 (Poulsen et al., 1986), no evidence was found for retroreduction of the hydroxylamine to the amine by human FMO3. It is likely that retroreduction is due to a CYP that has not been completely characterized (Clement et al., 1994; Kadlubar et al., 1973). There are several reports of the CYP-catalyzed retroreduction of hydroxylamines to amines (Clement et al., 1994), amidoximes to amidines (Clement et al., 1991) and N-hydroxyisothiourreas to isothiourreas (Clement, 1991). The retroreduction of N-hydroxydebrisoquine to debrisoquine (Clement et al., 1993) or N-hydroxyaminoquinidine to amino-guanidine (Clement et al., 1994) has been shown to be mediated by cytochrome b5, cytochrome b5 reductase and a CYP that is apparently similar to the retroreduction enzyme system described by Kadlubar and Ziegler (1974) for the retroreduction of hydroxylamines to amines. Parallel and interdependent oxidative and retroreductive enzymatic processes must be in action to account for the formation of oxime 3b and primary amine 1 from hydroxylamine 2 in the presence of hepatic microsomes.

Previously, some data from investigations using porcine FMO1 showed that direct N-oxygenation was not observed but, instead, porcine FMO1 released O2− in the presence of the hydroxylamine and converted the hydroxylamine nonenzymatically to the nitroxide (Rauckman et al., 1979). Uncoupling of porcine FMO1 hydroperoxylavin has been proposed to generate O2− or H2O2, and in a nonenzymatic step, autoxidize the hydroxylamines. Pig FMO1 has also been observed to form oximes from primary hydroxylamines (Clement et al., 1993) and to form nitrones from secondary hydroxylamines (Cashman et al., 1990). The formation of...
these two metabolites was judged to be strictly dependent on FMO action. A possible role for reactive oxygen species in the N-oxidation of phenethyl hydroxylamine 2 was examined by investigating the human microsomal and human FMO3-mediated production of oxime 3b in the presence of H2O2 and O2. Neither generation of O2 by the xanthine/xanthine oxidase system nor removal of O2 by SOD had any effect on the formation of oxime 3b from phenethylamine hydroxylamine 2 by cDNA-expressed human Lys158 FMO3-MBP (table 7). The conclusion is that in the presence of human liver microsomes or human FMO3, O2 and/or H2O2 contributes ≤3% to 4% of the oxime 3b formation from phenethylamine.

Human Lys158 FMO3-MBP did not reduce hydroxylamine 2 to phenethylamine 1, but in the presence of porcine or human liver microsomes, retroreduction of hydroxylamine 2 was significant and, paradoxically, reactive oxygen species apparently participated in primary amine formation. Thus, the O2 generating system of xanthine/xanthine oxidase increased by >2-fold the reduction of hydroxylamine 2 to primary amine 1 in the presence of adult human liver microsomes.

Restriction length polymorphism and oligonucleotide-sequencing studies showed that in humans, codon 158 of human FMO3 encoded either amino acids Glu or Lys at approximately equal allele frequencies for the Caucasian population examined. Although this may not be the case for all human populations, nevertheless, an investigation of the prominent forms of human FMO3 involved in biogenic amine metabolism is important. As shown in table 8, the K mapp value for N-oxidation of phenethylamine 1 and phenethylamine hydroxylamine 2 was 2.2- to 7.4-fold lower for the Glu158 FMO3 enzyme than for the wild-type Lys158 FMO3 enzyme, respectively. The V max/K mapp values also suggest that the human Glu158 FMO3 enzyme more efficiently N-oxidizes phenethylamine than the Lys158 FMO3 enzyme. In either case, however, the K mapp values are low enough to be of potential physiological relevance in metabolizing lipophilic phenethylamines to pharmacologically inactive metabolites.

The FMO has often been cited as a detoxication catalyst that converts lipophilic compounds into pharmacologically inert metabolites (Cashman, 1995; Ziegler, 1980). Most examples of FMO-mediated detoxication that have been studied have been xenobiotics because of the paucity of endogenous physiologically active materials that are substrates for FMO. In the case of the biogenic amine phenethylamine that potentially interacts with a number of pharmacological targets, we were able to investigate this hypothesis. As shown in table 9, no detectable affinity for any of the prominent biogenic receptors or transporters was observed for the hydroxylamine 2 or oxime 3. Thus, metabolism of phenethylamines to hydroxylamines or oximes should result in abrogation of pharmacological activity. It is possible that phenethylamine hydroxylamines or oximes are further metabolized by conjugation to other pharmacologically inert materials. Regardless, FMO may play a fundamental role in converting pharmacologically important biogenic amines to oxime metabolites that effectively terminate the pharmacological activity.

Acknowledgments

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