CYP2B6, CYP3A4, AND CYP2C19 ARE RESPONSIBLE FOR THE IN VITRO N-DEMETHYLATION OF MEPERIDINE IN HUMAN LIVER MICROSOMES

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ABSTRACT:

Meperidine is an opioid analgesic metabolized in the liver by N-demethylation to normeperidine, a potent stimulant of the central nervous system. The purpose of this study was to identify the human cytochrome P450 (P450) enzymes involved in normeperidine formation. In vitro studies included 1) screening 16 expressed P450s for normeperidine formation, 2) kinetic experiments on human liver microsomes and candidate P450s, and 3) correlation and inhibition experiments using human hepatic microsomes. After normalization by its relative abundance in human liver microsomes, CYP2B6, CYP3A4, and CYP2C19 accounted for 57, 28, and 15% of the total intrinsic clearance of meperidine. CYP3A5 and CYP2D6 contributed to <1%. Formation of normeperidine significantly correlated with CYP2B6-selective S-mephenytoin N-demethylation (r = 0.88, p < 0.0001 at 75 μM meperidine, and r = 0.89, p < 0.0001 at 350 μM meperidine, n = 21) and CYP3A4-selective midazolam 1'-hydroxylation (r = 0.59, p < 0.01 at 75 μM meperidine, and r = 0.55, p < 0.01 at 350 μM meperidine, n = 23). No significant correlation was observed with CYP2C19-selective S-mephenytoin 4'-hydroxylation (r = 0.36, p = 0.2 at 75 μM meperidine, and r = 0.02, p = 0.9 at 350 μM meperidine, n = 13). An anti-CYP2B6 antibody inhibited normeperidine formation by 46%. In contrast, antibodies inhibitory to CYP3A4 and CYP2C8/9/18/19 had little effect (<14% inhibition). Experiments with thiopeta and ketoconazole suggested inhibition of microsomal CYP2B6 and CYP3A4 activity, whereas studies with fluvoxamine (a substrate of CYP2C19) were inconclusive due to lack of specificity. We conclude that normeperidine formation in human liver microsomes is mainly catalyzed by CYP2B6 and CYP3A4, with a minor contribution from CYP2C19.

Meperidine is a synthetic opioid widely used for treating pain and controlling postanesthetic shivering (Clark et al., 1995; Simopoulos et al., 2002; Roy et al., 2004). Its main pharmacological action is produced through μ receptors on the central nervous system (CNS) and the neural elements in the bowel (Clark et al., 1995). Meperidine is metabolized extensively in the human liver by 1) N-demethylation to normeperidine (6-N-desmethylmeperidine) (Fig. 1), which may be further hydrolyzed to normeperidinic acid and subsequently conjugated (Plotnikoff et al., 1956), and 2) by hydrolysis to meperidinic acid by human carboxylesterase 1 followed by conjugation (Plotnikoff et al., 1956; Bourland et al., 1997; Zhang et al., 1999). Meperidine metabolites account for over half the drug in the urine (Zhang et al., 1999).

Normeperidine is devoid of analgesic activity, but it is a potent stimulant of the CNS (Clark et al., 1995). Its main adverse effect is neurotoxicity, producing symptoms that include nervousness, tremors, muscle twitches, and seizures (Clark et al., 1995; Simopoulos et al., 2002). Multiple doses of meperidine result in accumulation of normeperidine due to its long elimination half-life (15–30 h) as compared with the parent drug (2.4–4 h) (Clark et al., 1995). Since normeperidine is eliminated via the liver and kidney, patients with significant impairment of hepatic or renal function have prolonged normeperidine half-lives and are especially predisposed to its toxic effects (Clark et al., 1995; Simopoulos et al., 2002). Other individuals susceptible to the adverse effects of normeperidine include 1) individuals who receive prolonged administration of meperidine (such as cancer patients) or high meperidine doses (>400–600 mg/day), 2) patients receiving oral meperidine, 3) individuals treated with monoamine oxidase inhibitors, and 4) patients receiving medications that induce hepatic enzyme systems (Clark et al., 1995; Simopoulos et al., 2002).

ABBREVIATIONS: CNS, central nervous system; P450, cytochrome P450; HPLC, high-performance liquid chromatography; Clint, intrinsic clearance; CAR, constitutive androstane receptor; PXR, pregnane X receptor.
The aim of our study was to investigate the in vitro formation of normeperidine by human liver microsomes and to identify the P450 enzyme(s) involved in this reaction. The results of our experiments will help to predict clinically significant drug-drug interactions that may occur when drugs that alter P450 activity are coadministered with meperidine, affecting its pharmacological and toxicological properties.

Furthermore, identification of the enzymes responsible for normeperidine formation could potentially reduce the incidence of the serious neurotoxicity of this agent.

Materials and Methods

Chemicals and Reagents. Meperidine hydrochloride, diphenhydramine hydrochloride, ketoconazole, fluvoxamine, thiotepa, and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). S-Mephyton, nirvanol, and 4',5'-hydroxy-S-mephenytoin were supplied by BD Gentest (Woburn, MA). Normeperidine was purchased from Isotec Inc. (Miamisburg, OH). Midazolam and 1'-hydroxymidazolam were obtained from Hoffman-La Roche (Nutley, NJ). Methanol and acetonitrile (both HPLC grade) were purchased through NJ). Methanol and acetonitrile (both HPLC grade) were purchased through NJ. All other reagents and chemicals used were of the highest grade and commercially available.

Human Liver Microsomes. Human liver tissue was procured at St. Jude Children’s Research Hospital, and was provided by the Liver Tissue Procurement and Distribution System (Pittsburgh, PA; funded by National Institutes of Health Contract No1-3K-9-2310) and by the Cooperative Human Tissue Network.

Microsomes Expressing Human P450 Isoforms. Microsomes from cDNA-transfected baculovirus-insect cells expressing CYP1A1, CYP1A2, CYP2A6+b5, CYP1B1, CYP2B6+b5, CYP2C8+b5, CYP2C9+b5, CYP2C18, CYP2C19+b5, CYP2D6, CYP2E1+b5, CYP3A4+b5, CYP3A5, CYP3A7+b5, CYP4A11, and CYP19 (aromatase) were purchased from BD Gentest. All microsomes contained cDNA-expressed human P450 reductase. Control insect cell microsomes from wild-type baculovirus-infected cells were also obtained from BD Gentest.

Antibodies. Antibodies inhibitory to CYP2B6 (monoclonal, ascites-based), and CYP3A4 (polyclonal, raised in rabbits) were obtained from BD Gentest. A monoclonal antibody from mice ascites inhibitory against CYP2C8/9/18/19 (1-68-11) was purchased from Invitrogen (Carlsbad, CA). The lack of cross-reactivities of the immunoinhibitory antibodies to the P450 isoforms were demonstrated by the manufacturers (wwwbdbiosciences.com/discovery_labware/gentest, www.lifetechnologies.com). Confirmatory evidence of the specificity of the anti-CYP2B6, anti-CYP3A4, and anti-CYP2C8/9/18/19 antibodies was provided by studies by Roy et al. (1999), Shiraga et al. (1999), and Yang et al. (1999).

Incubations with Human Liver Microsomes. Preliminary experiments were performed to determine incubation conditions that both maximized normeperidine production and ensured linearity with respect to microsomal protein concentration and incubation time. A typical incubation contained 0.25 mg of human liver microsomes, meperidine (dissolved in incubation buffer), 1 mM NADPH, 5 mM MgCl2, and 100 mM sodium phosphate buffer (pH 7.4), in a final volume of 250 μL. After preincubation for 5 min at 37°C, the reaction was initiated by the addition of NADPH. The reaction was terminated after 30 min by adding 500 μL of cold methanol. Internal standard (40 μg/mL diphenhydramine, dissolved in methanol) was added to the samples before centrifugation (20 min, 14,000 rpm, 4°C). The supernatants were evaporated to dryness using nitrogen gas (37°C). Samples were reconstituted with 200 μL of mobile phase acidified with phosphoric acid (final acid concentration, 1 mM) and centrifuged (10 min, 11,000 rpm, 4°C). Aliquots of 175 μL were analyzed by HPLC. Control incubations were performed by omitting NADPH.

HPLC. Normeperidine formation was measured with a modification of the chromatographic method published by Netherall et al. (1985). The mobile phase consisted of 70% of a mix of 15 mM dipotassium hydrogen phosphate and methanol (pH 6.7) (83:17, v/v), and 30% acetonitrile, delivered at a flow rate of 1 ml/min. Separation was achieved with an LC-CN column (4.6 × 250 mm, 5 μm) (Supelco, Bellefonte, PA) and a NovaPak CN Guard-Pak insert (Waters, Milford, MA). The eluate was monitored using UV detection at 205 nm (L-4250 UV-VIS detector; Hitachi High Technologies America, San Jose, CA). The run time was 20 min. Retention times of normeperidine, meperidine, and diphenhydramine were 6.0, 6.6, and 10.4 min, respectively. Normeperidine production was quantified using a standard curve containing known amounts of normeperidine and internal standard. The quantitation limit of normeperidine was 2.1 μM. The intra-assay coefficients of variation (CVs) and accuracy were determined by performing five measurements of the same three normeperidine standards on the same day. At normeperidine concentrations of 30.3 μM, 11.6 μM, and 2.1 μM, the %CVs and accuracy were 2.4 and 99%, 4.1 and 100%, and 2.2 and 92%, respectively. Interassay reproducibility and accuracy were determined by performing assays of the same three standards in triplicate on three consecutive days. The percentages of CV and accuracy were 6.0 and 99%, 8.8 and 102%, and 8.7 and 92% at 40.3 μM, 11.6 μM, and 2.1 μM, respectively.

Screening of Recombinant Human P450 Isosforms for Normeperidine Formation. Incubations with P450s were performed as described for human hepatic microsomes, using 25 pmol of P450 and 85 μM meperidine. Control microsomes prepared from insect cells infected with wild-type baculovirus were used as negative controls to control for native enzyme activities. The incubations were performed in duplicate.

Kinetics Studies using Human Liver Microsomes and Recombinant P450 Enzymes. Kinetic parameters (apparent Km , Vmax, and intrinsic clearance) in pooled human liver microsomes (0.25 mg) or P450 enzymes (CYP2B6, CYP2C18, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) (25 pmol) were determined as described above using a meperidine concentration range of 0.0042 to 6.3 mM. The experiments with human liver microsomes were performed in triplicate, whereas the recombinant P450 experiments were done in duplicate.

Correlation Studies in Human Liver Microsomes. Human liver microsomal preparations (0.25 mg) were incubated with meperidine (75 μM and 350 μM) to test the level of correlation between the N-demethylation of meperidine and the activities of CYP2B6, CYP3A4, and CYP2C19. Incubations with 350 μM meperidine were performed as described above. For the incubations with 75 μM meperidine, the final incubation volume and the volume used for stop solution (100 mM sodium phosphate buffer) were increased to 500 μL and 1 ml, respectively; all other conditions were those described above. CYP2B6 activities (n = 21) were determined by measuring the formation of nirvanol from S-mephenytoin as described by Lamba et al. (2003). CYP3A4 activities (n = 23) were measured by the 1'-hydroxylation of midazolam as previously described (Relling et al., 1994). CYP2C19 activities (n = 13) were determined by analysis of the 4'-hydroxylation of S-mephenytoin using the incubation conditions and mobile phase phase published by Suzuki et al. (2002) and Ko et al. (1997), respectively. The analytes were separated using a μBondapak C18 column (3.9 × 300 mm, 10 μm) (Waters) and a NovaPak C18 Guard column (Waters). The flow rate was 1 ml/min. The compounds were detected at 211 nm (L-4250 UV-VIS detector; Hitachi High Technologies America). Under these conditions, 4-hydroxy-S-mephenytoin, 5-hydroxy-S-mephenytoin, and phenobarbital eluted at 7.7, 13.4, and 19.8 min, respectively. The experiments with midazolam were performed in triplicate. All other determinations were done in duplicate.

Inhibition Studies with Human Liver Microsomes and Recombinant P450 Enzymes. Pooled human liver microsomes (0.25 mg) or P450 enzymes (CYP2B6, CYP2C18, CYP2C19, and CYP3A4) (25 pmol) were preincubated with inhibitor for 5 min at 37°C and transferred to ice for 45 min. Incubations were conducted as described above using 100 μM meperidine. Thiotepa (50 μM and 10 μM, dissolved in incubation buffer), ketoconazole (2 μM and 1 μM, dissolved in methanol, 0.1% final methanol concentration in incubation mixture), and fluvoxamine (100 μM and 25 μM, dissolved in incubation buffer) were used for inhibition of CYP2B6 (Rae et al., 2002), CYP3A4 (Thummel and Wilkinson, 1998), and CYP2C19 (Rasmussen et al., 1998).
In the presence of NADPH, human liver microsomes metabolized conazole (contained no inhibitor). Experiments were performed in duplicate. Appropriate solvents (incubation buffer or 0.1% methanol in the case of ketoconazole) were used. CYP2B6, CYP2C19, and CYP3A4 microsomes were also coexpressed with human cytochrome b5.

**Materials and Methods**

Formation rates were determined using HPLC as described under Materials and Methods. Values represent the mean ± S.E. of an experiment performed in duplicate. The measured intrinsic clearances were normalized using nominal amounts of P450 content in human liver microsomes (Mankowski, 1999; Rodrigues, 1999; Kassahun et al., 2001). All P450 preparations contained cDNA-expressed human P450 reductase. CYP2B6, CYP2C19, and CYP3A4 microsomes were also expressed with human cytochrome b5.

Data Analysis and Statistics.

Activities were expressed as mean ± standard deviation (S.D.). Apparent $K_m$ and $V_{max}$ values were calculated by nonlinear regression and expressed as mean ± standard error (S.E.). To test whether the data followed Michaelis-Menten kinetics, Eadie-Hofstee plots were constructed. When visual inspection of Eadie-Hofstee plots suggested substrate activation or inhibition, the F test was used to compare the Michaelis-Menten model with alternative models (a Hill allosteric model in the case of substrate activation, or a Michaelis-Menten model with uncompetitive inhibition in the case of substrate inhibition) (Venkataraman et al., 1999). When the p value of the F test was less than 0.05, the alternative models were chosen for kinetic analysis. Intrinsic clearance ($CL_{int}$) was expressed as $V_{max}/K_m$. The measured intrinsic clearances were normalized using nominal amounts of P450 content in human liver microsomes (Mankowski, 1998; Rodrigues, 1999; Kassahun et al., 2001).

The Spearman correlation coefficient was used to test the level of correlation between the formation of normeperidine and P450 probe substrates. The threshold value for statistical significance was set at 0.05. The percentage of inhibition was expressed as the ratio of the amount of normeperidine formed in the presence of inhibitor to the control activity. Data and statistics analyses were done using GraphPad Prism 4.00 for Windows (GraphPad Software Inc., San Diego, CA; www.graphpad.com).

### Results

**N-Demethylation of Meperidine by Human Liver Microsomes.**

In the presence of NADPH, human liver microsomes metabolized meperidine to normeperidine. Normeperidine formation was not observed when NADPH was omitted from the incubations. The normeperidine formation rate (mean ± S.D.) in a set of 23 human liver microsomes was 227 ± 196 pmol/min/mg protein. The coefficient of variation was 74%. Eadie-Hofstee plots of the kinetic data obtained using pooled human liver microsomes (n = 17) exhibited a biphasic pattern. The high-affinity component had apparent $K_{m1}$ and $V_{max1}$ values of 45.4 ± 12.8 µM and 124 ± 17.0 pmol/min/mg protein, respectively. The low-affinity component exhibited apparent $K_{m2}$ and $V_{max2}$ values of 1240 ± 185 µM and 1230 ± 60 pmol/min/mg protein, respectively. The catalytic efficiency of the high-affinity component (2.73 µl/min/mg protein) was approximately 3 times higher than that observed for the low-affinity component (0.99 µl/min/mg protein).

**N-Demethylation of Meperidine by Recombinant P450s.**

Figure 2 shows the results of a screening experiment to determine which P450s are capable of catalyzing normeperidine formation. CYP2B6 exhibited the highest rate of meperidine N-demethylation. Other enzymes catalytically active in normeperidine formation included CYP2C19 > CYP2C18 > CYP3A4 > CYP2D6. Minor normeperidine production (<1.8 mg/min/pmol P450) was observed with CYP1A1, CYP1A2, CYP2C8, CYP3A5, CYP3A7, and CYP4A11 and 19. Production of normeperidine was not observed with CYP2A6, CYP1B1, CYP2C9, CYP2E1, and insect control microsomes.

Kinetic parameters were determined using the five isoforms with the highest catalytic activity for normeperidine formation, and CYP3A5 (chosen due to its polymorphic nature; Kuehl et al., 2001) (Table 1). Formation of normeperidine by CYP2B6 and CYP2D6 followed Michaelis-Menten kinetics. Convex Eadie-Hofstee plots of CYP3A4- and CYP3A5-catalyzed normeperidine formation suggested substrate autoactivation (data not shown). Comparison of the Michaelis-Menten and Hill allosteric models gave p values of 0.2 and <0.05 for CYP3A4 and CYP3A5, respectively. Consequently, the CYP3A4 and CYP3A5 kinetic data were analyzed using a Michaelis-Menten and Hill allosteric model, respectively. The Hill coefficient for the reaction catalyzed by CYP3A5 was 1.2 ± 0.1 (mean ± S.E.). CYP2C19 and CYP2C18 showed apparent substrate inhibition at meperidine concentrations above 2 mM (data not shown). Their kinetic data best fitted a sigmoidal Michaelis-Menten model with uncompetitive inhibition when compared with the Michaelis-Menten (hyperbolic) model (p < 0.0001, F test).

CYP2B6 exhibited the highest rate of meperidine N-demethylation, approximately five times higher than that of CYP2C19, CYP2C18 and CYP3A4. When compared with CYP2B6, turnover rates 15- and 30-times lower where observed with CYP3A5 and CYP2D6, respec-

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**Table 1**

<table>
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<tr>
<th>Microsomes</th>
<th>$K_m$</th>
<th>$V_{max}$</th>
<th>$CL_{int}$</th>
<th>Nominal P450 Content</th>
<th>Adjusted $CL_{int}$</th>
<th>Percentage Adjusted $CL_{int}$</th>
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</thead>
<tbody>
<tr>
<td>CYP2B6</td>
<td>356 ± 34</td>
<td>138 ± 3</td>
<td>0.388</td>
<td>39</td>
<td>15.1</td>
<td>57</td>
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<tr>
<td>CYP3A4</td>
<td>355 ± 81</td>
<td>25.0 ± 1.3</td>
<td>0.070</td>
<td>108</td>
<td>7.56</td>
<td>28</td>
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<tr>
<td>CYP2C19</td>
<td>131 ± 12</td>
<td>26.9 ± 0.9</td>
<td>0.205</td>
<td>19</td>
<td>3.90</td>
<td>15</td>
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<tr>
<td>CYP2D6</td>
<td>446 ± 71</td>
<td>4.62 ± 0.35</td>
<td>0.010</td>
<td>10</td>
<td>0.100</td>
<td>&lt;1</td>
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<tr>
<td>CYP3A5</td>
<td>977 ± 63</td>
<td>9.21 ± 0.24</td>
<td>0.009</td>
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<td>0.009</td>
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<tr>
<td>CYP2C18</td>
<td>356 ± 47</td>
<td>26.4 ± 1.7</td>
<td>0.074</td>
<td>&lt;2.5</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not determined.

* Data fitted using a Michaelis-Menten model.

* Data fitted using a Michaelis-Menten model with uncompetitive inhibition (p < 0.0001, F test).

* Data fitted using the Hill allosteric model (p < 0.05, F test; Hill coefficient = 1.2 ± 0.1).

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**Fig. 2.** N-Demethylation of meperidine by expressed human P450 isoforms. Incubations were performed using 85 µM meperidine, 25 pmol of P450 microsomal protein, and 1 mM NADPH for 30 min at 37°C. Formation rates were measured by HPLC as described under Materials and Methods. Each bar represents the mean (pmol of normeperidine/min/pmol P450) of duplicate determinations. ND means nondetectable activity.
The lowest Km value was observed with CYP2C19 followed by CYP3A4
and CYP2B6.

To better assess the relative contribution of the individual P450 isoforms to the in vitro formation of meperidine in human liver microsomes, the intrinsic clearances were normalized according to the nominal levels of the P450s as determined by immunoblotting (Mankowski, 1999; Rodrigues, 1999; Kassahun et al., 2001). CYP2B6 accounted for more than half (57%) the adjusted intrinsic clearance, followed by CYP3A4 (28%) and CYP2C19 (15%). The adjusted intrinsic clearances of CYP2D6 and CYP3A5 were negligible (<1%). The adjusted intrinsic clearance of CYP2C18 could not be determined due of its low nominal content in human liver microsomes (<2.5 pmol/mg) (Rodrigues, 1999).

**Correlation Studies in Human Liver Microsomes.** Based on the enzyme kinetic results, we proceeded to test the level of correlation between the N-demethylation of meperidine and the activity of CYP2B6, CYP3A4, and CYP2C19 enzymes using probe substrates. We did not perform correlation studies with CYP2C18 because, to the best of our knowledge, no specific CYP2C18 substrates have been identified. A high level of correlation was found between the formation of normeperidine and nirvanol (r = 0.88, p < 0.0001 at 75 μM meperidine, and r = 0.89, p < 0.0001 at 350 μM meperidine, n = 21) (Fig. 3, A and B). The formation rates of normeperidine and 1'-hydroxymidazolam were significantly correlated (r = 0.59, p < 0.01 at 75 μM meperidine, and r = 0.55, p < 0.01 at 350 μM meperidine, n = 23) (Fig. 3, C and D). No significant association was observed between the N-demethylation of meperidine and the 4'-hydroxylation of S-mephenytoin (r = 0.36, p = 0.2 at 75 μM meperidine, and r = 0.02, p = 0.9 at 350 μM meperidine, n = 13) (Fig. 3, E and F).

**Inhibition Studies.** To test the effect of inhibitors on the N-demethylation of meperidine by human liver microsomes, we performed experiments with immunoinhibitory antibodies and P450 isoform-specific chemical inhibitors. We first performed experiments with recombinant P450s and immunoinhibitory antibodies to confirm inhibition of the individual isoforms. Formation of normeperidine by CYP2B6, CYP3A4, and CYP2C19 microsomes was decreased by 46%, 46%, and 82%, respectively, using the respective antibodies. In pooled human liver microsomes, the antibody against CYP2B6 inhibited the formation of normeperidine by 46%, whereas minor inhibition (<14%) was observed after incubation with antibodies inhibitory to CYP3A4 and CYP2C8/9/18/19 (data not shown).

We chose chemical inhibitors for CYP2B6, CYP3A4, and CYP2C19 based on the literature (Rasmussen et al., 1998; Thummel and Wilkinson, 1998; Rae et al., 2002). Chemical inhibition of CYP2C18 was not investigated since we do not know of any specific inhibitor of this isoform. Figure 4 shows a decrease in the in vitro production of normeperidine from meperidine after addition of thiotepa, ketoconazole, and fluvoxamine to human liver microsomes. To investigate the specificity of the chemical inhibitors to each isoform, we also performed experiments with the four P450s of interest (Fig. 4). Thiotepa reduced the catalytic activities of CYP2B6 and CYP3A4.
performed with 100 percentages of control incubations in the absence of inhibitor. Incubations were CYP2C19 isoforms. The rates of normeperidine formation were expressed as human liver microsomes and recombinant CYP2B6, CYP3A4, CYP2C18, and the mean of two determinations.

Ketoconazole inhibited the enzymatic activities of mainly CYP3A4, but inhibition of CYP2C18 was also observed. Fluvoxamine reduced the activities of not only CYP2C19 and CYP2C18, but also CYP3A4 and CYP2B6.

**Discussion**

Meperidine has been in clinical use for over 50 years. Despite the direct association between the accumulation of its N-demethylated metabolite and susceptibility to CNS toxicity, there have been no studies investigating the P450 enzymes responsible for the formation of normeperidine. The results of our experiments demonstrate the main involvement of CYP2B6 and CYP3A4 in the in vitro N-demethylation of meperidine in human liver microsomes. CYP2C19 seems to be also involved, although at a lesser capacity than CYP2B6 and CYP3A4. We reached this conclusion based on evidence from experiments to determine the capacity of individual P450s for producing normeperidine, correlation studies with P450 probe substrates, and inhibition experiments. Evidence of the role of CYP2B6 in the in vitro formation of normeperidine was first observed in screening experiments and enzyme kinetic studies. Our work showed CYP2B6 as the isoform with the highest metabolic capacity for normeperidine formation, accounting for 57% of the total intrinsic clearance. Correlation studies demonstrated a highly significant association between the N-demethylation of both meperidine and (S)-mephenytoin (an indicator of CYP2B6 activity) in human liver microsomes, indicating that both metabolites are produced by the same enzyme. Inhibition experiments showed an immunoinhibitory antibody against CYP2B6 as the most potent inhibitor of meperidine N-demethylation in human liver microsomes. In addition, thiotepa (a CYP2B6 substrate) inhibited the formation of normeperidine by human liver microsomes, suggesting competitive inhibition of CYP2B6 activity. CYP3A4 also appears to significantly contribute to the in vitro formation of normeperidine by human liver microsomes. We concluded this upon observing that 1) after normalization by its relative abundance in human liver microsomes, CYP3A4 was responsible for 28% of the total intrinsic clearance, and 2) normeperidine formation in hepatic microsomes was modestly correlated with CYP3A4-specific activity and inhibited by ketoconazole. Kinetic parameters obtained with CYP2C19 microsomes (lowest in all enzymes and a contribution of 15% to the total normalized intrinsic clearance) demonstrated that this enzyme contributes to normeperidine formation. However, since correlation experiments with human liver microsomes did not reach statistical significance and due to the low expression levels of CYP2C19 in the human liver (Lapple et al., 2003), we believe that the involvement of CYP2C19 in the formation of meperidine is less than that of CYP2B6 and CYP3A4. We could not draw any conclusions from the inhibition experiments with fluvoxamine since, under our experimental conditions, it lacked specificity as a CYP2C19 inhibitor.

The maximum formation rate ($V_{\text{max}}$) obtained in CYP2C18 kinetic experiments closely resembled that of CYP2C19. We could not perform correlation and chemical inhibition experiments with CYP2C18 due to the lack of specific probe substrates and inhibitors for this enzyme. We do not consider this to be a limitation of our study since CYP2C18 protein has not been found in detectable amounts in human hepatic tissue or culture hepatocytes (Gerbal-Chaloin et al., 2001; Lapple et al., 2003), and this enzyme has not been shown to be of clinical importance (Goldstein, 2001). The low formation rates and metabolic clearances of CYP3A5 and CYP2D6 suggest that these isoforms do not play an appreciative role in normeperidine formation, although these rates might be underestimated due to the lack of cytochrome $b_5$ in their microsomal preparations.

The minimum analgesic concentration of meperidine in blood is 1.8 $\mu$M (Clark et al., 1995). Due to constraints imposed by the sensitivity of our assay, we could not quantify reliably the meperidine N-demethylation activities at such substrate concentration. Our studies were performed at meperidine concentrations higher than the pharmacologically relevant levels, and we recognize that this is a limitation of our study. Correlation and inhibition experiments were performed using concentrations of meperidine of 75 $\mu$M and 100 $\mu$M, respectively. We believe that these concentrations, although higher than the $K_m$ in human liver microsomes (45 $\mu$M), still represent high-affinity microsomal enzyme activity. Correlation experiments were also performed at a higher substrate concentration (350 $\mu$M). We recognize that results obtained at this higher concentration might represent a combination of high and low enzyme activity. Nevertheless, the correlation results obtained at this higher concentration, especially for CYP2B6 and CYP3A4, closely resemble those obtained at 75 $\mu$M meperidine. In the case of CYP2C19, the correlation between the formation of normeperidine and the 4'-hydroxylation of
S-mephenytoin improved at 75 μM meperidine, although it still failed to reach statistical significance even though the \(K_m\) of CYP2C19 (131 μM) is closer to 75 μM than to the \(K_m\) values of CYP2B6 (356 μM) and CYP3A4 (355 μM).

The biphasic kinetics of the meperidine \(N\)-demethylation reaction in human liver microsomes suggested that this reaction is catalyzed by a minimum of two enzymes with significantly different (high and low) substrate affinities. We hypothesize that CYP2B6 and CYP3A4 represent high-affinity activity and that CYP3A5, with a \(K_m\) in the millimolar range, probably contributes to the low-affinity component observed in human liver microsomes. CYP2B6 followed traditional Michaelis-Menten kinetics. Apparent substrate inhibition, observed with CYP2C19 and CYP2C18, has been previously observed in P450 studies (Venkatarkrishnan et al., 2001). This behavior is characterized by a decrease in metabolic rate at high substrate concentrations. On the other hand, the kinetics of CYP3A5 suggested cooperative binding of meperidine to the enzyme (coautocatysis of CYP3A5 upon meperidine binding). Instances of positive cooperativity have been observed for several CYP3A4-mediated reactions, suggesting that the active site may accommodate two molecules, and these molecules influence the kinetics of each other (Venkatarkrishnan et al., 2001). Although preliminary examination of our CYP3A4 kinetic results suggested possible allosteric kinetics, the data best fit the Michaelis-Menten model.

Meperidine possesses essential structural characteristics (capability of interaction with enzyme binding sites through hydrophobic interactions and hydrogen bonding) that are common in CYP2B6, CYP3A4, and CYP2C19 substrates (Payne et al., 1999; De Rienzo et al., 2000; Wang and Halpert, 2002; Lewis, 2003). CYP2B6 and CYP3A4 are known to metabolize lipophilic compounds with the site of oxidation often constituted by nitrogen atoms (De Rienzo et al., 2000; Wang and Halpert, 2002). For example, CYP2B6 catalyzes the \(N\)-demethylation of amitriptyline, dextromethorphan, diazepam, imipramine, codeine, lidocaine, and ethyl-morphine (De Rienzo et al., 2000).

CYP3A4, CYP2B6, and CYP2C19 are involved in the metabolism of many clinically important drugs (Venkatarkrishnan et al., 2000). CYP3A4 is the most abundant P450 enzyme in the human liver. CYP2B6 appears to be expressed at higher levels than previously thought, according to expression data generated with selective and sensitive CYP2B6 antibodies (Ekins et al., 1998). A study by Venkatarkrishnan et al. (2000) reported the CYP3A, CYP2B6, and CYP2C19 content in human liver microsomes to be 3.6 to 732.5 pmol/mg microsomal protein \((n = 12)\), 1.5 to 148.4 pmol/mg microsomal protein \((n = 12)\), and 3.1 to 24.9 pmol/mg microsomal protein \((n = 10)\), respectively. Considering the relative expression levels of these three enzymes, our results suggest that CYP3A4 and CYP2B6 are very likely to be clinically important when administering meperidine. CYP2C19 seems to play a lesser role in the formation of normeperidine when compared with CYP3A4 and CYP2B6.

CYP2B6 is expressed in all human livers and is the most important P450 in drug metabolism. CYP2B6 expression levels exhibit large interindividual differences which might be due to CYP2B6 genetic polymorphisms (Lang et al., 2001; Jinno et al., 2003; Xie et al., 2003), variability in the expression levels of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) genes (Chang et al., 2003), and environmental factors such as alcohol and nicotine consumption (Miksys et al., 2003). Genetic polymorphisms associated with reduced hepatic CYP2B6 activity may decrease clearance of meperidine and elevate its plasma concentrations. Variants associated with increased CYP2B6 activity might reduce the therapeutic efficacy of meperidine and increase the risk of developing neurotoxicity due to increased normeperidine levels.

Since transcriptional activation of CYP3A4 and CYP2B6 is regulated by similar mechanisms (Pascussi et al., 2003), we expect that coadministration of meperidine with drugs capable of altering or binding to CAR and PXR (such as phenobarbital and phenytoin) might affect the therapeutic efficacy, disposition, and safety of meperidine. Drug interactions between meperidine and both phenobarbital and phenytoin have been documented (Stambaugh et al., 1977, 1978; Pond and Kretschmar, 1981). Concomitant use of meperidine and either one of these drugs has been associated with decreased meperidine excretion, increased normeperidine excretion, and enhanced sedation. Since phenobarbital and phenytoin are inducers of CYP3A4 and CYP2B6 activity (Martin et al., 2003), these observations are consistent with our observation that CYP3A4 and CYP2B6 play a significant role in meperidine metabolism to normeperidine. Other individuals that may be predisposed to experiencing neurotoxicity due to increased levels of normeperidine include smokers and alcoholics, since these subjects have higher levels of brain CYP2B6 (Miksys et al., 2003), and meperidine is distributed to the brain.

Since it is likely that meperidine is coprescribed with metabolic inducers or inhibitors, our findings may have considerable clinical importance. Our results provide the basis for future studies investigating the clinical relevance of CYP2B6 and CYP3A4 genetic polymorphisms and drug interactions on the efficacy, disposition, and toxicity of meperidine.

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References


