Aromatase Is the Major Enzyme Metabolizing Buprenorphine in Human Placenta

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ABSTRACT

Buprenorphine (BUP) is a partial opiate agonist used for treatment of the adult and the pregnant addicted to this class of narcotics. The kinetic parameters for transplacental transfer and the metabolism of BUP during its perfusion in a placental lobule were the subject of an earlier report from our laboratory. The aim of this investigation is to identify and characterize the enzyme catalyzing the metabolism of BUP in term human placenta. Norbuprenorphine (norBUP) is the only metabolite formed as determined by high performance liquid chromatography and mass spectrometry. The activity of the enzyme responsible for BUP metabolism is highest in the microsomal fraction and lowest in the cytosolic, with the mitochondrial in between. Compounds with selective affinity to the enzyme aromatase (CYP 19), namely 4-hydroxyandrostenedione and aminogluthethimide, caused >70% inhibition of norBUP formation. Monoclonal antibodies raised against CYP 19 were the most potent inhibitors of BUP dealkylation. A comparison between the data obtained from the saturation isotherm for BUP dealkylation by placental microsomes and a commercially available system of cDNA-expressed CYP 19 indicated similar kinetic parameters, with apparent $K_m$ values of 12 ± 4.0 and 14 ± 8.0 μM, respectively. Therefore, aromatase is the major enzyme catalyzing the biotransformation of BUP to norBUP in term human placentas obtained from healthy pregnancies. The minor involvement of other cytochrome P450 isoforms or enzyme(s) in the metabolism of BUP in placental tissue cannot be ruled out.

Methadone is, and has been for decades, the drug of choice in treatment of the adult and the pregnant opiate addict. In the last decade, data obtained from clinical trials on treatment of the pregnant woman with BUP indicated that the drug was well tolerated by the patient and that the incidence of neonatal abstinence was low to nonexistent (Marquet et al., 1997; Fischer et al., 2000; Eder et al., 2001), suggesting that BUP might be an alternative to methadone for maintenance of this patient population.

BUP levels in the fetal circulation can have a direct effect on its normal growth and development. On the other hand, maternal circulating levels of the opiate could affect placental physiology and, consequently, the fetus. The kinetics for transplacental transfer of BUP, its concentration in the fetal circuit, and effects on placental functions were determined in our laboratory utilizing the technique of dual perfusion of placental lobule (Nanovskaya et al., 2002). Our data indicated that the tissue accumulated 13 times the amount of BUP present in the maternal circuit, and less than 5% of it was metabolized to norBUP. However, in the perfusion system mentioned, BUP was delivered in the intervillous space, thus bypassing the maternal myometrium and endometrium, and its access to the metabolizing enzymes was less. Therefore, it is likely that in vivo metabolism of BUP in the placenta is higher.

In humans, BUP is biotransformed to norBUP by hepatic enzymes during first pass metabolism. The major enzyme responsible for N-dealkylation of BUP to norBUP in the liver is cytochrome P450 3A4 (CYP 3A4) (Iribarne et al., 1997; Kobayashi et al., 1998). The formation of the glucuronide conjugates of BUP and norBUP was also reported (Cone et al., 1984). During pregnancy, the human placenta plays an important role in the metabolism of endogenous compounds, xenobiotics, and environmental pollutants (Juchau 1980; Contractor, 1983; Nandakumaran et al., 1983, Blanck et al., 1983; Roe et al., 1990; Pienimaki et al., 1997). The expression and activity of various P450 isoforms depends on placental gestation age and tissue maturity but is lower than that in the liver (Hakkola et al., 1996a,b). Aromatase/CYP 19 is one of the cytochrome P450 isozymes with a well established role in the biosynthesis of steroids in human placenta (Thompson

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ABBREVIATIONS: BUP, buprenorphine; norBUP, norbuprenorphine; P450, cytochrome P450; EFC, 7-ethoxy-4-trifluoromethylcoumarin; HFC, 7-hydroxy-4-trifluoromethylcoumarin; LC/MS, liquid chromatography/mass spectrometry; HPLC, high-performance liquid chromatography; ANOVA, analysis of variance.
and Siiiteri, 1974). More recently, the role of aromatase in the oxidative metabolism of xenobiotics by preparations of crude and purified enzymes has been reported (Toma et al., 1996; Osawa et al., 1997). These data were further substantiated by the availability of high specific activity cDNA-expressed CYP 19 that allowed testing the metabolism of several compounds and determining their kinetic parameters (McNamara et al., 1999).

The above underscores the importance of identifying the enzyme responsible for BUP metabolism in human placenta and the effects of prolonged administration of the opiate on its activity. The information obtained is necessary to avoid drug interactions between other therapeutics that may be administered during utilization of BUP for treatment of the pregnant opiate addict. Therefore, the focus of this investigation is to identify and characterize the enzyme responsible for BUP metabolism in term human placenta.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise mentioned. Acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ); BUP and norBUP were a gift from the National Institute on Drug Abuse drug supply unit.

Clinical Material

All placentas were obtained from term healthy pregnancies after delivery according to a protocol approved by the Institutional Review Board. Every effort was made to exclude placentas of women who abused drugs during pregnancy.

Villous tissue was dissected, rinsed with ice-cold saline, and homogenized in 0.1 M potassium phosphate buffer pH 7.4 (Ultra Turrax, Staufen, Germany). The homogenate was used to prepare subcellular fractions by differential centrifugation; namely, 10,000 g pellet for the mitochondrial, 104,000 g pellet for the microsomal, and the supernatant for the cytosolic fraction. The mitochondrial and microsomal pellets were resuspended in 0.1 M potassium phosphate buffer (pH 7.4), and their protein content was determined (Bio-Rad kit, Hercules, CA) using bovine serum albumin as a standard. Aliquots from the subcellular fractions were stored at −80°C until used.

Other

All monoclonal antibodies were a generous gift from Dr. Andrew Parkinson (Xenotech LLC, Lenexa, KS). Rabbit antiserum to human placental aromatase was obtained from Hauptman-Woodward Institute (Buffalo, NY). The effect of these antibodies on placental aromatase activity was reported earlier (Kitawaki et al., 1989). The cDNA-expressed CYP 19 system is commercially available as Supermatase activity was reported earlier (Kitawaki et al., 1989). The characterization of Supermats indicated that its activity. The information obtained is necessary to avoid drug interactions between other therapeutics that may be administered during utilization of BUP for treatment of the pregnant opiate addict. Therefore, the focus of this investigation is to identify and characterize the enzyme responsible for BUP metabolism in term human placenta.

Enzyme Reactions

Monooxygenases. The activity of these enzymes was determined by the O-deethylation of the substrate 7-ethoxy-4-trifluoromethylcoumarin (EFC) and formation of the fluorescent product 7-hydroxy-4-methylpyrazole. The reaction volume of 1 ml was incubated for 10 min before the substrate EFC was added, and in the second, all compounds were added simultaneously.

N-Dealkylation of BUP. The activity of placental mitochondrial, microsomal, and cytosolic fractions in catalyzing the N-dealkylation of BUP to norBUP was determined. The reaction volume was made of 1 ml of 0.1 M potassium phosphate buffer (pH 7.4) and contained 1 mg protein of the subcellular fraction, 50 μM BUP. The components were preincubated for 5 min at 37°C followed by addition of the NADPH-regenerating system that started the reaction period of 30 min. The latter was made of: 0.4 mM NADP, 4 mM glucose-6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, and 2 mM MgCl2. The reaction period was terminated by the addition of 100 μl of a 35% (w/v) trichloroacetic acid containing 1 μg/ml terfenadine (internal standard) and centrifuged at 18,000 g for 10 min. The amount of norBUP formed in the supernatant was determined by liquid chromatography/mass spectrometry as described below. The control was identical, but the placental fraction was denatured. The dependence of the activity of the microsomal fraction (1 mg/ml) and preparations of human cDNA expressing CYP 19 (40–100 pmol of CYP 19/ml) (Gentest) on the amount of BUP (saturation curve) was determined.

Aromatase (CYP 19) Activity. The activity of aromatase in the mitochondrial and microsomal fraction was determined by its conversion of testosterone to 17β estradiol. The following was preincubated for 5 min at 37°C: 250 μl of protein and 1.0 μM testosterone in a final volume of 1 ml of 0.1 M potassium phosphate buffer. The reaction was initiated by the addition of NADPH-regenerating system, and the incubation continued for another 5 min. It was then terminated by the addition of 100 μl of 10% (w/v) trichloroacetic acid followed by 100 μl of 10 μg/ml estrone as an internal standard. The reaction solution was centrifuged at 12,000 g for 10 min, the pellet was discarded, and estradiol formed was determined in the supernatant.

Inhibition of norBUP Formation

Identification of the P450 enzyme catalyzing the metabolism of BUP to norBUP was achieved by utilizing the following inhibitors: chemicals, i.e., compounds selective for specific P450 isoforms; and monoclonal antibodies against purified human liver P450 isoforms.

Chemical Inhibitors. The concentration range used for each inhibitor was based on its IC50, Ks, or Km values for a specific P450 isoform. The following are the inhibitors, the concentration used, and their corresponding P450 isoforms: a-naphthoflavone, 0.1 μM (CYP 1A); sulfaphenazole, 10 μM (CYP 2C); quinidine, 5 μM (CYP 2D6); 4-methylpyrazole, 50 μM (CYP 2E1); ketoconazole, 2.5 μM (CYP 3A4/CYP 19); trolox and amnoglutethimide 10 μM (CYP 19) (Stresser et al., 2000). Concentrated stock solutions of the inhibitors in methanol were prepared, and an aliquot of each was used to attain the final concentration required for each P450 isofrom as specified above. Each inhibitor, BUP at a final concentration of 12 μM (=Km), and the microsomal preparation in potassium phosphate buffer (1 mg of protein/ml) were preincubated for 5 min at 37°C. The reaction was initiated by the addition of the NADPH-regenerating system and incubated for a period of 30 min. The control reaction contained all the above-mentioned components but with 0.5% (v/v) methanol substituting for the inhibitor solution. The effect of 2.5 μM ketoconazole was investigated in commercially available systems expressing either CYP 3A4 or CYP 19 under identical conditions.

Monoclonal Antibodies. Monoclonal antibodies against human liver CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4/5 and rabbit antiserum to human placental aromatase were utilized to identify/confirm the P450 enzyme responsible for BUP metabolism by placental tissue. A pool of microsomal preparations obtained from 12 placentas was utilized.
In each enzyme assay, 0.1 mg of protein of the microsomal pool was incubated at room temperature with an antibody at its concentration causing 90% inhibition of the P450 isofrom it was raised against. The microsomal pool and the antibodies were incubated for 15 min before BUP was added to attain a final concentration of 50 μM, and the reaction was allowed to continue for another 120 min at 37°C before it was terminated as described above. Mouse IgG replaced the monoclonal antibodies in the control reaction.

Quantitative Determination of the Reaction Products

7-Hydroxy-4-trifluromethylcoumarin (HFC). The concentration of HFC was determined by fluorescence spectroscopy (CytoFluor, Series 4000 Fluorescence; Applied Biosystems, Foster City, CA) according to the method of Buters et al., (1993). A standard curve for fluorescence intensity of 20 to 2000 pmol/ml HFC, with excitation and emission wavelengths of 450 and 530 nm, respectively, was plotted. The fluorescence intensity at zero time of the incubation period served as a blank.

BUP and norBUP. BUP and norBUP were identified by HPLC according to the method of Iribarne et al. (1997) and as described earlier in detail (Nanovskaya et al., 2002).

Liquid Chromatography (LC) and Mass Spectrometry (MS) Analysis

The amounts of norBUP formed in the reaction mixture were determined utilizing LC/MS. A Spectra system consisting of an AS 3000 autosampler, P 4000 pump (Spectra Physics, San Jose, CA) and a 2 × 30 mm C-18 3-μm Luna column (Phenomenex, Torrance, CA) was used. The mobile phase was made of a linear gradient of acetonitrile/water starting with 10% of the former and ending with 90% at a flow rate of 500 μl/min for a period of 5 min. The ratio was then reverted to 10% acetonitrile for 1 min before the end of the run. The volume of the injected sample was 100 μl. The eluant of the column was coupled to AQA single-quadrupole Navigator LC/MS (Thermo Quest, San Jose, CA). Mass spectral analyses were performed using electrospray in the positive mode with an ionizing voltage of 4.0 kV, and is attributed to interplacental variations. Therefore, the reaction mixture was determined by use of nonlinear regression analysis with SPSS Version 11 for Windows (SPSS Science, Chicago, IL). The data were fit to the Michaelis-Menten equation: \( v = \frac{(V_{max} \times S)}{K_m + S} \). Statistical analysis of data on the effect of inhibitors on BUP metabolism was carried out using one-way ANOVA with Tukey’s comparison and deemed significant if the p value was <0.05.

Results

Activity of Placental Monoxygenases. Villous tissue of term human placentas (n = 10) was used to prepare the subcellular fractions. The activity of each fraction in catalyzing the deethylation of the marker substrate EFC (50 μM) to HFC was determined. The formation of HFC, under saturating concentrations of EFC, was proportional to the deethylation activity of the subcellular fractions. The mean activity for EFC O-deethylation by the placental subcellular fractions is shown in Table 1. A wide range of values for the deethylation activity of the microsomal fractions activity was observed, with the highest being almost 15 times the lowest, and is attributed to interplacental variations. Therefore, the deethylation activity was arbitrarily divided into two groups, below and above 40 pmol/mg protein • min, and are referred to as “low activity” and “high activity,” with one preparation reaching 300 pmol/mg protein • min in the latter. On the other hand, a smaller range of values for the activity of the enzyme(s) catalyzing the dealkylation of BUP to norBUP was observed in all the subcellular fractions. These differences did not reach statistical significance (at p < 0.05); i.e., no interplacental variations were observed (Table 1).

The activities of placental mitochondrial and microsomal fractions in catalyzing the dealkylation of BUP and the marker substrate EFC were compared. The ratio of mitochondrial to microsomal EFC O-deethylation was equal to 1, and that for N-dealkylation of BUP was 0.5 (Table.1). These data indicate that the dealkylation of BUP by placental mi-

<p>| TABLE 1 |
|-----------------|------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Substrate</th>
<th>EFCOD Activity</th>
<th>Microsomal Fraction</th>
<th>Mitochondrial Fraction</th>
<th>Cytosolic Fraction</th>
<th>Mean Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFC</td>
<td>High</td>
<td>190.9 ± 101.7</td>
<td>154.2 ± 65.4</td>
<td>1.5 ± 0.8</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>12.4 ± 3.9</td>
<td>14.1 ± 5.5</td>
<td>2.01 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>BUP</td>
<td>N.A.</td>
<td>1.9 ± 0.6</td>
<td>1.1 ± 0.4</td>
<td>0.2 ± 0.05</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

EFCOD, EFC O-deethylation; N.A., not applicable.
crosomal fractions was more active than that by the mitochondrial fractions. Minor contamination of the microsomal fraction with mitochondrial enzymes cannot be ruled out, but a similar observation has been reported earlier (Pasanen et al., 1985) and is addressed further in determination of aromatase activity utilizing its natural substrate, testosterone.

The effect of BUP (10–200 μM) on EFC deethylase was investigated and its IC_{50} was determined (Fig. 1). The inhibition was concentration-dependent and was only observed in the placental preparations in the high activity group. The IC_{50} values for BUP in the mitochondrial and microsomal fractions were 129.2 ± 61.1 and 129.4 ± 75.9 μM, respectively. The inhibitory effect of BUP was observed whether the opiate was added before or simultaneously with EFC to the reaction mixture.

**Kinetic Parameters for BUP Dealkylation.** Our data suggest that the enzyme catalyzing the dealkylation of BUP to norBUP may be present in the three subcellular fractions of placental tissue, with the microsomal fraction having the highest activity. Therefore, a pool of microsomal fractions prepared from six placentas (1–6, Table 2) was utilized to determine the apparent \( K_m \) and \( V_{max} \) for the dealkylation of BUP. The enzyme catalyzing the reaction required NADPH. Analysis of the saturation curves for the biotransformation of BUP to norBUP revealed an apparent \( K_m \) of 12 ± 4.0 μM, a \( V_{max} \) of 2.9 ± 0.7 pmol/mg protein · min, and an intrinsic clearance (\( V_{max}/K_m \)) value of 0.3 ± 0.1 μl/mg protein · min (Table 2; Fig. 2).

**The Effect of Inhibitors Selective for P450 Isoforms on BUP Metabolism.** Inhibitors selective for P450 isoforms were utilized to identify the enzyme catalyzing the dealkylation of BUP to norBUP. Ketoconazole (2.5 μM), 4-hydroxyandrostenedione (1.0 μM), and aminoglutethimide (10.0 μM) caused the highest inhibition (70% of control). \( \alpha \)-Naphthoflavone and 4-methylpyrazole caused 20% and 40% inhibition at their respective concentrations of 0.1 and 50 μM (Fig. 3). The least amount of inhibition (20%) was caused by 5 μM quinidine, whereas sulfaphenazole and troleandomycin did not show any inhibition of BUP metabolism in the concentration range tested.

Ketoconazole is considered an inhibitor of hepatic CYP 3A but is also known to inhibit human placental aromatase (Ayub and Levell, 1988). The effect of ketoconazole on norBUP formation by cDNA preparations expressing CYP 3A4 and CYP 19 was investigated. Ketoconazole (2.5 μM) caused a 90% inhibition of BUP metabolism in both preparations; i.e., it was as effective an inhibitor of CYP 19 as it was of CYP 3A4. Taken together, our data suggest that the human placental microsomal enzyme catalyzing N-dealkylation of BUP to norBUP could be CYP 19.

**The Effect of Monoclonal Antibodies on BUP Metabolism.** Monoclonal antibodies raised against specific human liver P450 isoforms were used to confirm the identification of the enzyme catalyzing BUP metabolism. A pool of microsomal preparations from 12 placentas was utilized in these experiments. Each monoclonal antibody raised against a specific P450 isoform was tested at its concentration causing 80% inhibition. The highest inhibition observed for BUP metabolism (70%) was observed in the presence of the antibodies raised against CYP 19. Monoclonal antibodies against CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and CYP 3A4/5 did not cause any significant inhibition of norBUP formation (Fig. 4). Data obtained on the effects of the selective inhibitors and monoclonal antibodies on the enzyme-catalyzed dealkylation of BUP indicate that it is CYP 19/ aromatase.

**Kinetic Parameters for norBUP Formation by cDNA-Expressed CYP 19.** The cDNA-expressed CYP 19 isozyme (Supersomes) catalyzed the dealkylation of BUP to norBUP. Analyses of the saturation curves obtained revealed apparent

**TABLE 2**

<table>
<thead>
<tr>
<th>Microsomal Preparation No.</th>
<th>( K_m ) (mM)</th>
<th>( V_{max} ) (pmol/mg protein · min)</th>
<th>Intrinsic Clearance (( V_{max}/K_m )) (μl/mg protein · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.7</td>
<td>4.1</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>6.8</td>
<td>2.0</td>
<td>0.29</td>
</tr>
<tr>
<td>3</td>
<td>8.5</td>
<td>2.8</td>
<td>0.33</td>
</tr>
<tr>
<td>4</td>
<td>14.5</td>
<td>2.8</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>13.6</td>
<td>2.7</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>16.2</td>
<td>3.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean</td>
<td>11.6</td>
<td>2.9</td>
<td>0.27</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.7</td>
<td>0.7</td>
<td>0.09</td>
</tr>
</tbody>
</table>
**Discussion**

Buprenorphine is an opiate agonist used for treatment of the adult addicted to this class of narcotics; it is well tolerated by the pregnant woman, and the incidence of neonatal abstinence is minimal to nonexistent (Jarvis and Schnoll, 1994; Marquet et al., 1997; Fischer et al., 2000; Lejeune et al., 2001). A recent report from our laboratory indicated that BUP transfer to the fetal circuit is low when administered into the maternal circuit of the dually perfused placentallobe; it accumulates in the tissue and is metabolized to norBUP during the experimental period (Nanovskaya et al., 2002). This report provides data on identification and characterization of the human placental enzyme responsible for the metabolism of BUP to norBUP.

Microsomal CYP 3A4 is the enzyme responsible for the biotransformation of 75% of BUP to norBUP during first-pass metabolism. The remaining 25% is metabolized by CYP 3A5, 3A7, and 2C8, but other minor products may also be formed by CYP 2C18, 2C19, 2D6, and 2E1 (Iribarne et al., 1997; Kobayashi et al., 1998, Moody et al., 2002).

Our working hypothesis was that the enzyme responsible for BUP metabolism in the placenta was an oxygenase. Accordingly, the oxygenase activity of placental subcellular fractions was determined by utilizing the EFC O-deethylation to the fluorescent product HFC (DeLuca et al., 1988; Buters et al., 1993). Two distinct forms of monoxygenases catalyze this reaction in placental tissue; namely, a constitutive form that includes CYP 19/aromatase (Meigs, 1987) and an inducible CYP 1A1 (Pasanen et al., 1990). The determined EFC O-deethylase activity in placental subcellular fractions revealed a wide range of values for \( V_{\text{max}} \) (Table 1) and was arbitrarily divided into two groups: low activity, < 40 pmol/mg protein · min, and the remainder as high activity. A similar observation for O-deethylation activity was reported earlier; the high activity group of placentas was associated with maternal smoking during pregnancy and has been attributed to induction of CYP 1A1 (Pasanen et al., 1990). On the other hand, the activity of our placental preparations in the \( N \)-dealkylation of BUP did not reflect the wide range of variability observed for the O-deethylation of EFC (Table 1). Since the term placentas utilized in this investigation were obtained from women who may have been smokers, the involvement of CYP 1A1 in BUP metabolism was excluded.

The highest activity for the enzyme catalyzing the metabolism of BUP to norBUP was in the microsomal fraction (Table 1). The reaction required NADPH and exhibited sat-
urokinase kinetics with an apparent $K_m$ of 12 $\mu$M and $V_{max}$ of 2.9 pmol/mg protein · min (Fig. 2; Table 2). An Eadie-Hofstee plot of the data revealed monophasic kinetics indicating that either a single enzyme or multiple enzymes with similar affinities catalyze the reaction. The values obtained for intrinsic clearance (Table 2) ranged between 0.18 and 0.42 $\mu$l/mg protein · min (mean $\pm$ 0.27) and is considerably lower than that reported for human liver, 17 $\mu$l/mg protein · min (Kobayashi et al., 1998). These data suggest that human liver intrinsic clearance (Table 2) ranged between 0.18 and 0.42 $\mu$l/mg protein · min (mean $\pm$ 0.27) and is considerably lower than that reported for human liver, 17 $\mu$l/mg protein · min (Kobayashi et al., 1998). These data suggest that human liver enzymes. The most potent inhibitors of norBUP formation were ketoconazole, 4-hydroxyandrostenedione, and aminoglutethimide (Fig. 3), suggesting that CYP 3A4 and CYP 19 could be the enzymes catalyzing the reaction. In our investigations, ketoconazole caused a 70% inhibition of norBUP formation (Fig. 3) at its concentration of 2.5 $\mu$M that is selective for its effect on CYP 3A4 (Fabre et al., 1993; Baldwin et al., 1995; Pelkonen et al., 1998). Ketoconazole also inhibited the metabolism of BUP by two cDNA preparations expressing CYP 3A4 and CYP 19. Therefore, our data confirmed earlier reports that ketoconazole is an inhibitor of CYP 19 (Ayub and Levell, 1988), with $K_i$ values similar to those of hepatic microsomal CYP 3A4 (Stresser et al., 2000). A role for CYP 3A4 as the enzyme catalyzing the metabolism of BUP by placental microsomal preparations was excluded because troleandomycin, a selective inhibitor of the isozyme (Newton et al., 1995), had no effect on norBUP formation. Taken together, our data on the effects of chemical inhibitors of the activity of placental microsomes on BUP metabolism suggest that CYP 19 is the enzyme catalyzing the reaction.

Monoclonal antibodies raised against CYP 19 were the most potent inhibitor of norBUP formation, confirming the profile of the chemical inhibitors and the conclusion drawn (Fig. 4). Moreover, analysis of the kinetics for BUP metabolism by a cDNA-expressed CYP 19 revealed a $K_m$ value similar to that obtained for the placental enzyme (Fig. 5).

The activity exhibited by the mitochondrial fraction in metabolizing BUP and our direct determination of testosterone aromatization by CYP 19 in both subcellular fractions are in agreement with earlier reports on the presence of aromatase activity in the mitochondria (Finkelstein et al., 1985; Pasanen et al., 1985).

Our finding that CYP 19 is the enzyme responsible for the metabolism of BUP in human placenta raises the issue of drug interactions during pregnancy. Aromatase/CYP 19 is the enzyme responsible for the conversion of androgens to estrogens and the metabolism of certain xenobiotics in human placenta (Meigs, 1987; Toma et al., 1996; McNamara et al., 1999). It is unclear whether BUP metabolism by placental microsomes would interfere with the biosynthesis of these hormones or the detoxification of other drugs.

In summary, data in this report provided evidence that aromatase/CYP 19 is the enzyme catalyzing the metabolism of BUP to norBUP in trophoblast tissue obtained from term human placentas.

Acknowledgments

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References


Buprenorphine Metabolism in Human Placenta


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