Transplacental Transfer and Metabolism of Buprenorphine

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ABSTRACT

Information on the direct and indirect effects of buprenorphine (BUP) on the fetus is essential for determining its potential for treatment of the pregnant opiate addict. The goal of this investigation is to determine the transplacental transfer of BUP to the fetal circulation, its metabolism, and effects on the tissue. The technique of dual perfusion of placental lobule was used. The range of BUP concentrations investigated included its peak plasma levels (10 ng/ml) in patients under treatment. A biphasic decline in concentration of the drug in the maternal circulation was observed, initially rapid then slow. During the initial (60 min), the tissue sequestered most of BUP resulting in a low (<10%) transplacental transfer of the drug to the fetal circulation. The concentration ratios of the drug in tissue/maternal and tissue/fetal were 13 ± 6.5 and 27.4 ± 0.4. The drug sequestered did not have any adverse effects on placental tissue viability and functional parameters. Less than 5% of the perfused BUP was metabolized to norbuprenorphine during the 4 h of perfusion and the metabolite was distributed between the tissue, maternal, and fetal circulations. Taken together, these data suggest that the therapeutic levels of BUP in the maternal circulation may have no indirect effects (via the placenta) on the fetus. The observed low transplacental transfer of BUP to the fetal circuit may explain the moderate/absence of neonatal withdrawal in the limited number of reports on mothers treated with the drug during pregnancy.

For several decades methadone has been the drug of choice for treating the pregnant opiate-dependent patient and numerous reports cited its successes or limitations, and a review of that literature would be out of the scope of this report. However, it should be pointed out that researchers in the field agreed that the development of alternative drugs is necessary. Indeed, two decades of research on treatment of drug abuse lead to 13 principles of which the first states “No single treatment is appropriate for all individuals” (Mathias, 1999). Buprenorphine (BUP) and L-α-acetylmethadol were identified for treatment/maintenance therapy of the adult opiate addict. The aim of this investigation is to provide the information necessary to determine the potential of BUP for treatment of the pregnant opiate addict.

BUP has been suggested as an alternative for maintenance therapy of opiate-dependent subjects because it produced limited withdrawal symptoms, resulted in reduced heroin self-administration, and had a longer duration of action (Jasinski et al., 1978; Mello and Mendelson, 1980). Several reports confirmed the safety and efficacy of BUP in treatment of the opiate-dependent adult (Johnson et al., 1992; Ling et al., 1996, 1998; Fischer et al., 1999). However, the very limited number of reports available on the maternal and neonatal outcome of BUP maintenance therapy of the pregnant opiate addict indicates that it is well accepted by the patient and the incidence of neonatal abstinence syndrome is mild to nonexistent (Marquet et al., 1997; Fischer et al., 2000).

BUP is a semisynthetic, highly lipophilic opiate derived from thebaine with a molecular weight of 504.1. Its binding to fractions of α- and β-globulins is very high but is insigificant to albumin (USP Drug Information, 1999). Reports on the agonist/antagonist properties of BUP indicate that it is either a partial agonist or an agonist/antagonist at the μ- and an agonist at the κ-receptors (Paul et al., 1992; Pick et al., 1997). Its binding to human placental κ-opiate receptors and their mediated responses as well as its effects on the tissue are currently unknown.

BUP is metabolized in human liver by its N-dealkylation to the pharmacologically active norbuprenorphine, which along with the parent compound is conjugated with glucuronic acid (Cone et al., 1984). Hepatic microsomal cytochrome P450 3A4 (CYP3A4) is responsible for metabolizing most (75%) of buprenorphine, whereas other enzyme(s), yet to be identified, are responsible for the remaining 25% (Iribarne et al., 1997; Kobayashi et al., 1998). Human placentas obtained from uncomplicated pregnancies has the potential of expressing several CYP genes, including the CYP3A family, but the amount of its pro-

ABBREVIATIONS: BUP, buprenorphine; CYP, cytochrome P450; hCG, human chorionic gonadotropin; AP, antipyrine; AUC, area under the concentration-time curve; norBUP, norbuprenorphine; HPLC, high-performance liquid chromatography.
tein in the tissue appears to be extremely low (Hakkola et al., 1996a,b; Pasanen, 1999).

Drugs administered to patients during pregnancy may have direct and or indirect effects on the fetus. The majority of the latter are the result of the adverse effects of a drug on placental functions responsible for normal fetal growth and development. On the other hand, the direct effects of a drug can result from its concentration in the fetal circulation, which depends, largely, on its transfer and metabolism by placental tissue. Data available on the transfer of drugs across human placenta and their metabolism by the tissue in vivo are restricted to those obtained at the time of delivery and do not reflect the dynamic state of concentration changes. Animal experiments offer a limited alternative because of the complexity and diversity of human placenta from that of any other species. However, the technique of in vitro dual perfusion of term human placental lobule has proven a valuable tool for providing data on the kinetics of transplacental transfer of numerous drugs, their effects on the tissue, and their metabolism (Wier et al., 1983; Schneider, 1995; Boal et al., 1997; Pienimaki et al., 1997).

We report here on the kinetics of transplacental transfer of BUP, its metabolism, and effects on term human placenta obtained from uncomplicated pregnancies.

**Materials and Methods**

**Dual Perfusion of Placental Lobule.** All the experimental conditions followed in our laboratory are identical to those described by Miller et al. (1993) and are briefly outlined below.

Each placenta was obtained from a full-term uncomplicated pregnancy as determined by one of the obstetrics staff in the Labor and Delivery Ward of Truman Medical Center (Kansas City, MO). Any evidence of maternal infection, systemic disease, and drug or alcohol abuse during pregnancy excluded the placenta from this study. All placentas were obtained according to an approved protocol by the Institutional Review Board.

**Experimental Protocol.** After visual inspection of the peripheral cotyledons for tears, two chorionic vessels (one artery and one vein) were cannulated with 3F and 5F umbilical catheters, respectively. The trimmed cotyledon was placed in the perfusion chamber with the maternal surface upward. The intervolus space on the maternal side was perfused by two catheters piercing the basal plate. A large venous drain was connected to a peristaltic pump, which continuously removed the fluid from the chamber and either returned it to the maternal reservoir (closed system) or to a separate container (open system). The perfusate was made of tissue culture medium M 199 (Invitrogen, Carlsbad, CA) containing dextran 40 (7.5 g/l in the maternal and 30 g/l in fetal perfusate), 1 g/l glucose, 25 IU/ml heparin, 40 mg/l gentamicin sulfate, 80 mg/l sulfamethoxazole, and 16 mg/l trimethoprim. A solution of 7.5% (w/v) sodium bicarbonate was used to adjust the pH to 7.4. Albumin binds BUP, its metabolism, and effects on term human placenta obtained from uncomplicated pregnancies.

**Transplacental Transfer and Distribution of BUP.** The adverse effects of BUP on the perfused placental tissue were determined by adding BUP to the maternal perfusate (referred to as transfusate) to achieve the final concentrations of 0.5, 2.5, 10, 15, and 30 ng/ml. The range of BUP concentration investigated included its peak serum levels in patients under treatment (Walsh et al., 1994; Chawarski et al., 1999) as well as in patients who suffered from toxicity due to its CO administration with benzodiazepines (Traquci et al., 1998). Samples (650 μl) were collected from the perfusates during each experiment, centrifuged at 1000g for 10 min at 4°C, and the supernatant stored at −70°C until the concentrations of glucose, lactate, and human chorionic gonadotropin (hCG) were determined. Glucose utilization, an indicator of tissue metabolic activity, was determined by the Glucose Trinder kit (Sigma Chemical, St. Louis, MO). Lactate production, an indicator of hypoxia or ischemia, hyperoxygenation, and normoxic conditions, was determined by the Lactate reagent kit (Sigma Chemical). The concentration of hCG, an indicator of the tissue functionality, was determined by an IRMA kit (Diagnostics Production Corp., Los Angeles, CA). In vitro hCG release from explant cultures of different placentas covers a wide concentration range (Cemeric et al., 1991) and reflects the variability in maternal serum levels of the hormone between individuals during pregnancy (Althann and Stenman, 1990), i.e., an intrinsic property of the tissue. Therefore, levels of hCG released during the control were set at 100 and those during the experimental release as percentage of the former.

In each experiment, the values obtained for these parameters during the initial 2-h control period represented baseline levels and were compared with those obtained during the following experimental period of 4 h during which BUP was transfused. Samples (110 μl) were collected every 15 min from both circuits and immediately analyzed for pH, pO2, and pCO2 by using a blood gas analyzer (Radiometer, Copenhagen, Denmark) (Wier and Miller, 1985).

For histology evaluation, the perfused cotyledons were fixed in formalin and prepared for light microscopy examination according to an established procedure (di Sant’Agnese et al., 1987).

**Transplacental Transfer and Distribution of BUP.** The kinetic parameters for transplacental transfer of BUP were determined by using different modes of the perfusion system, namely, the maternal and fetal perfusates were recirculated (closed-closed); the maternal was closed and the fetal open (closed-open), and both circuits were not recirculated (open-open) (Johnson et al., 1995). Each of the five doses of BUP investigated was added to the maternal reservoir to achieve a final concentration range between 0.5 and 30 ng/ml. Antipyrine (AP; 20 μg/ml) was used as marker for the transfer of an inert compound across the placental tissue and as a reference to account for interplacental variations. The radioactive isotopes [3H]BUP (16.36 Ci/mmol) was prepared by the Research Triangle...
Institute (a gift from the National Institute on Drug Abuse) and [14C]AP (9.3 mCi/mmol; Sigma Chemical) were added to the maternal reservoir (1.5 μCi of each) to increase the detection limits of the two compounds and to allow for their simultaneous determination by scintillation counting. Samples, each of 0.65 ml, were collected from the maternal and fetal perfusates at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, and 240 min. The concentrations of BUP and AP were determined in 0.5-ml aliquots of the perfusates by using a liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL). The results obtained represent the transfer of free drugs because no proteins (e.g., albumin) were added to the perfusates.

The open-open mode of perfusion was used to determine the transfer rates of AP and BUP and was calculated according to the following equations (Bourget et al., 1985): fetal transfer rate (TRF) = [CFV/CMa] × 100 (%), where CFV and CMa are venous fetal and arterial maternal concentrations of the opiate; maternal clearance (CLm) = (CFV/CMa) × QF, (ml/min), where QF is the fetal perfusion rate; and clearance index (Clindex) = TRF/BUP/TPR/AP.

To investigate the extent to which the placenta acts as a depot for BUP, the drug and AP were transfused in the maternal reservoir at a “steady-state concentration” (open circuit) for the initial 2 h of the experimental period. The maternal reservoir was then replenished with fresh medium devoid of the two drugs and the perfusion was continued for another 2 h under the same conditions, “washing period”. Samples were collected from both circuits during the experimental period of 4 h and their concentrations analyzed as described above.

The closed-open mode of perfusion was used to determine the elimination half-life (t1/2) and the elimination rate constant. The area under the concentration–time curve (AUC) for the maternal circulation was determined in our laboratory for control placentas (Table 1). These values were pooled and their radioactivity determined using a liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL). The rates of BUP distribution between the maternal, fetal, and tissue compartments were determined in a series of experiments that were terminated after either 1, 2, or 4 h. Samples from the maternal and fetal reservoirs were collected during perfusion and their content of BUP determined by a liquid scintillation counter as described above. At the end of each experiment, the perfused region (white) was cut from adjoining tissue, weighed, and approximately 1 g was minced and incubated in 1 ml of 1 M NaOH for 12 h in the dark to allow for luminescence decay. The samples were then counted in 8 ml of scintillation cocktail.

Metabolism of Buprenorphine. Buprenorphine is formed by the N-dealkylation of the cyclopropyl methyl group. Because the radioactive isotope of BUP is labeled with tritium at C15 to C16, the norBUP formed retains the tritium label. Extraction of BUP and norBUP from placental tissue, maternal, and fetal perfusates was carried out as described by Irizarie et al. (1997). Briefly, the two compounds were extracted with diethyl ether (3 × 5 ml) and the organic phase evaporated by a stream of nitrogen at 40°C. The dried residue was dissolved in 0.5 ml of the HPLC mobile phase made of acetonitrile/water (30:70, v/v) containing 0.5% (v/v) triethylamine.

The pH of the mobile phase was adjusted to 3 by using orthophosphoric acid. The stationary phase was a C18 column and the retention time for BUP and norBUP standards were identified at a flow rate of 1 ml/min. Elution was monitored at A = 210 nm and the fractions corresponding to the retention times of BUP and norBUP were pooled and their radioactivity determined using a liquid scintillation spectrometer. The HPLC system used was a Varian Star system (Varian, Palo Alto, CA) consisting of a Varian 9021 solvent module, an autosampler model 9095, and an ultraviolet/visible detector connected in series with a Schoeffel Instrument model 970 fluorescence detector (McPherson, Elsevier-Biosoft, Cambridge, England) attached to a Hewlett Packard model 3395 integrator.

Mass spectrometry was used to quantitate norBUP when needed. The liquid chromatography/mass spectrometry instrument consisted of the following: HP 1100 binary pump; Finnigan TSQ-700 mass spectrometer; digital Unix workstation; Finnigan software ICIS, version 8.3; PerkinElmer autosampler; and an Eppendorf TC-50 column heater. Chromatographic separation was achieved on a 2 × 30 mm C18 3-μm Luna column (Phenomenex, Torrance, CA) at 40°C. A binary gradient system at 400 μl/min consisted of a mobile phase A made of deionized water/5% acetonitrile and a mobile phase B made of 95% acetonitrile/5% deionized water. Buffer was added to both mobile phases (5 ml of glacial acetic acid and 1.2 ml of ammonium hydroxide/liter of mobile phase). A linear gradient was used and started with 5% B and ended with 95% over a period of 3 min. The mass spectrometer parameters were as follows: ionization mode, positive electrospray; manifold pressure, 2 × 10−6 torr; electrospray ionization spray voltage, 4.4 kV; current, −10 μA; capillary temperature, 200°C; electron multiplier, 1600 V; collision-induced dissociation argon gas pressure, 1.7 mTorr and offset −18.0 V; and injection volume, 20 μl and split ratio 1:3.

Opiate Receptors Assay. A 5% fraction of placental villous tissue homogenate was prepared as described previously (Ahmed et al., 1981). Aliquots of the homogenate containing 250 μg of protein were placed in disposable glass tubes (13 × 100 mm) and the following was added to each tube: increasing amounts of [3H]BUP (saturation curve) and 50 mM Tris, pH 7.4, buffer to a final volume of 0.5 ml. Nonspecific binding was determined in presence of 1 μM levophanol. The tubes containing the binding assay components were incubated at 37°C for 45 min. The incubation period was terminated by rapid filtration through glass fiber filters (#32; Schleicher & Schuell, Keene, NH) presoaked in polyethylenimine by using a cell harvester (Brandel Inc., Gaithersburg, MD). The filters were dried under a heat lamp, placed in scintillation vials, cocktail fluid added, and radioactivity determined. The Ke and Vmax values for the specific binding of BUP to placental tissue x-receptors were determined by analysis of the data with a software program by G. A. McPherson (Elsevier-Biosoft, Cambridge, England).

Statistical Analysis. Each concentration of BUP tested was repeated in at least five placenta unless otherwise indicated. All values reported are expressed as mean ± S.E.M. or S.D. Statistical significance of the differences observed between BUP-treated and control placentas and between the control and experimental periods for each placenta were calculated by two-tailed t test. One-way repeated measures analysis of variance was applied to calculate statistical significance in continuous measurements as in the effect of the drug on placental viability and functional parameters with time of perfusion. Calculations were carried out using the software program SYSTAT for Macintosh (version 5.2).

Results

Biochemical, Physiological, and Morphological Evaluation of Placental Tissue. In all experiments, a dose of BUP between 125 and 7500 ng was added to the maternal reservoir to achieve a final concentration range of 0.5 to 30.0 ng/ml. At all the five tested concentrations, there was no detectable difference between fetal arterial and venous flow rates and the fetal arterial pressure never exceeded 40 mm Hg, indicating vascular integrity of the perfused lobule during the 4-h experimental period. The values for pH and partial pressure of gases remained within the normal range, i.e., those obtained during the control period and in control placentas. The difference in pH between arterial and venous remained >60 mm Hg, indicating adequate maternal-fetal perfusion overlap. Oxygen transfer from the maternal to fetal circulation ranged between 0.359 ± 0.17 and 0.540 ± 0.14 ml/min · kg and was not affected by the transfused BUP. The metabolic activity of the transfused lobule was assessed by its glucose utilization, oxygen consumption, and lactate production (viability parameters). All the values for these biochemical parameters remained within those for the initial control period as well as being similar to those determined in our laboratory for control placentas (Table 1). These
data indicate that BUP had no adverse effects on the viability parameters of the tissue.

The release of the trophoblast tissue-specific hormone hCG during transfusion of BUP is used as an indicator of placental function and a decline in its rate suggests an adverse effect. The rate of hCG release during the control period was set at 100% and that during the experimental as a percentage of it. An apparent gradual increase in release of the hormone, although not statistically significant, was observed with the increase in the concentration of BUP transfused (Table 1). However, a pronounced and statistically significant ($p < 0.01$) stimulation of hCG release was observed at the BUP concentration of 30 ng/ml (60 nM). Data in Table 1 indicate that BUP had no adverse effects on a physiological function of placental tissue. Morphological examination of the tissue by light microscopy did not reveal any structural differences between the tissue transfused with BUP and that of control placentas.

**Binding of Buprenorphine to Placental $\kappa$-Opiate Receptors.** The specific binding of $[^{3}H]$BUP to the $\kappa$-opioid receptors of the $P_2$ fraction prepared from placental villus tissue homogenates was investigated. A saturation curve for the specific binding of $[^{3}H]$BUP was constructed from experiments using a concentration range between 0.1 and 8.0 nM in presence or absence of 1 $\mu$m naxalone. Scatchard analysis of the saturation isotherms revealed $K_d$ and $B_{\text{max}}$ values of $0.83 \pm 0.23$ nM and $79.0 \pm 25.0$ fmol/mg of protein, respectively. These data indicate high-affinity binding of BUP to placental $\kappa$-receptors and an interplacental variation in the $\kappa$-opioid receptor density.

**Metabolism of Buprenorphine.** The biotransformation of $[^{3}H]$BUP to norBUP during its transfusion into the placental tissue was determined in a closed-closed system to allow accumulation of the metabolite. A dose of 2500 ng (10 ng/ml) supplemented with 1.5 $\mu$Ci of $[^{3}H]$BUP was added to the maternal reservoir and transfused for 4 h. In these experiments, AP was not CO transfused with BUP because its retention time was within a few seconds of that for norBUP in the HPLC system used for identification of the metabolite. Quantitative determination of the metabolite was carried out by liquid scintillation counting and confirmed by mass spectrometry when needed. Under our chromatographic conditions the average retention times for standard samples of norBUP and BUP were 6.7 and 17.2 min (Fig. 1A). The samples from tissue extracts, maternal, and fetal perfusates were chromatographed and the amount of tritium determined in each of the eluted fractions. The amount of norBUP formed was less than 5% of the perfused BUP and is distributed between the three compartments (Fig. 1, B–D). The quantity of norBUP was confirmed by mass spectroscopy according to standard chromatograms analyzed at $m/z$ 414.2 with a retention time of 1.83 min.

**Transplacental Transfer of Buprenorphine and Antipyrine.** In the experiment described below, BUP and AP were CO transfused at concentrations of 10 ng/ml and 20 $\mu$g/ml supplemented with 1.5 $\mu$Ci of the $[^{3}H]$- and $[^{14}C]$-isotopes, respectively.

The transfer of drugs with a molecular weight less than 1000 Da across human placenta depends on their physico-chemical properties but can be influenced by circulatory factors. Our experiments were conducted under constant flow rates for both circuits and were similar to those in vivo, thus the data reported here reflect the transfer of BUP across term placentas obtained from healthy individuals. A patient with a condition that affects uteroplacental blood flow such as kidney disease, high blood pressure, or preeclampsia may affect the in vivo transfer of BUP across the placenta.

The decline in AP concentration in the maternal circuit was accompanied by its simultaneous appearance in the fetal circuit (Fig. 2A). The concentration of AP in the fetal circuit at the end of the experiment reached $9.93 \pm 0.68$ $\mu$g/ml, i.e., $49.9 \pm 1.6\%$ of its initial concentration, indicating that the tissue retained negligible amounts of the drug. Because the recirculating perfusion system used (closed-closed) allows a drug to accumulate in the fetal circulation, equilibrium for AP between the two circuits was achieved within 2 h.

The other hand, the decline in the concentration of BUP in the maternal circulation exhibited a biphasic pattern, rapid during the initial 60 min and slower during the following 3 h. The concentration of BUP in the fetal circuit at the end of the experiment was $0.88 \pm 0.14$ ng/ml, which represents $8.6 \pm 1.3\%$ of its initial concentration in the maternal circuit (Fig. 2B). Accordingly, the rapid decline in the concentration of BUP in the maternal circuit during the initial 60 min can be attributed to its uptake and retention by the tissue rather than its transfer to the fetal circuit.

The low transplacental transfer of BUP from the maternal to fetal circuit was further demonstrated by the area under the time-concentration curve with an AUC fetal/maternal ratio of $0.29 \pm 0.007$ compared with $0.95 \pm 0.06$ for AP. In addition, the AUC for BUP increased with the increase in its dose, indicating

### Table 1

Table of the tested concentration range of BUP on the viability parameters of the tissue during the experimental period of 4 h relative to the initial 2-h control period in which the tissue was perfused with medium only.

<table>
<thead>
<tr>
<th>Concentration of BUP (ng/ml)</th>
<th>hCG Release</th>
<th>O$_2$ Consumption</th>
<th>Glucose Consumption</th>
<th>Lactate Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of Control Period</td>
<td>Experimental Period</td>
<td>% of Control Period</td>
<td>Experimental Period</td>
</tr>
<tr>
<td></td>
<td>ml/min/kg</td>
<td></td>
<td></td>
<td>$\mu$mol/min/g</td>
</tr>
<tr>
<td>Control placentas</td>
<td>88 ± 10.2</td>
<td>4.06 ± 1.60</td>
<td>94.8 ± 17.6</td>
<td>0.393 ± 0.09</td>
</tr>
<tr>
<td>0.5</td>
<td>87 ± 16.9</td>
<td>3.12 ± 1.20</td>
<td>88.3 ± 14.1</td>
<td>0.304 ± 0.11</td>
</tr>
<tr>
<td>2.5</td>
<td>103 ± 13.1</td>
<td>3.40 ± 1.11</td>
<td>109.6 ± 12.2</td>
<td>0.302 ± 0.08</td>
</tr>
<tr>
<td>10.0</td>
<td>114 ± 42.2</td>
<td>3.90 ± 0.52</td>
<td>104.9 ± 27.0</td>
<td>0.286 ± 0.07</td>
</tr>
<tr>
<td>15.0</td>
<td>N.D.</td>
<td>3.87 ± 0.60</td>
<td>102.3 ± 22.4</td>
<td>0.402 ± 0.07</td>
</tr>
<tr>
<td>30.0</td>
<td>187 ± 60.8 **</td>
<td>3.31 ± 0.63</td>
<td>97.7 ± 16.6</td>
<td>0.361 ± 0.06</td>
</tr>
</tbody>
</table>

N.D., not determined.

** Release of hCG varies widely between placentas in vivo and in vitro. Therefore, the amount of the hormone released from each placenta during the control period was set at 100% and that released during the experimental period as a percentage of it.
linear pharmacokinetics in the concentration range tested and suggesting (Fig. 3) that BUP is transported by passive diffusion.

The lag time, fetal transfer rate, and clearance of BUP were determined using the model system in its open-open mode, i.e., under steady-state conditions. The appearance of BUP and AP in the fetal circuit was rapid with similar lag times, indicating that the two drugs cross the placenta at approximately the same rate. However, BUP transfer to the fetal circuit was only a fraction (29%) of that observed for AP (Table 2). A comparison between the concentrations of the two drugs going in and out of the tissue during the initial 2 h (Fig. 4, A and B) illustrates a difference between the concentrations of AP in the maternal artery and vein, which can be accounted for by its appearance in the fetal vein. On the other hand, the concentration of BUP in the fetal vein does not account for the corresponding difference between that in the

Fig. 1. Retention times of norBUP (7.0 min) and BUP (17.0 min) standards in the HPLC system used (A). Identification of the fractions corresponding to norBUP and BUP in extracts of the maternal circuit (B), fetal circuit (C), and perfused tissue (D).

Fig. 2. A, concentrations of antipyrine in the maternal and fetal circuits during 4 h of perfusion in a closed-closed system. Equilibrium was reached after 90 min with a ratio of 1. B, concentrations of BUP in the maternal and fetal circuits during 4 h of perfusion. The amounts of BUP appearing in the fetal circuit correspond to a fraction of its decrease in the maternal circuit. Inset is an expanded y-axis to illustrate the concentration of BUP in the fetal circuit.

Fig. 3. Illustration of the linear relation between the dose of BUP administered and area under the curve for the maternal circuit.
Buprenorphine Transfer and Metabolism/Human Placenta

The amounts of BUP appearing in the fetal circuit during the 1st 2 h of perfusion under identical conditions to those mentioned for AP in A. B, concentrations of BUP in the maternal artery and vein. These data suggest BUP but not AP has been retained by the perfused lobule/tissue. After BUP and AP were transfused under steady-state conditions for 2 h (Fig. 4, A and B), the maternal reservoir was replenished with fresh medium and the experiment continued for another 2 h (washing period). The concentration of AP declined rapidly in the maternal and fetal circuits and was not detectable after 40 min. On the other hand, BUP exhibited a biphasic rate, initially (10 min) rapid in both circuits and was followed by a very slow and shallow decline in its concentration for the remaining 110 min. The concentration of BUP stabilized at approximately 40% of its level at the beginning of the washing period (Fig. 4B). These data indicate that BUP is gradually released from the tissue to the maternal and fetal veins over a period of 2 h, whereas AP was completely eliminated from the tissue within 40 min.

Rate of BUP Accumulation in Placental Tissue. The rate of BUP accumulation in placental tissue was determined by a series of experiments in which a dose of 2500 ng of BUP (10 ng/ml) was transfused in the maternal circuit for 1, 2, or 4 h (Fig. 5). After 1 h, 44.7 ± 6.01% of BUP initial dose was retained by the tissue and only 3% appeared in the fetal circulation. After 2 h, a slight increase in the amount of BUP retained by the tissue was observed (46 ± 5.62). After 4 h, the amount of BUP retained by the tissue was 58.8 ± 3.95% of its initial dose. Therefore, the rate of BUP accumulation in the tissue was highest during the 1st hour of transfusion and represented 78% of that after 4 h. The concentration ratios of BUP in the tissue/maternal and tissue/fetal were 13.1 ± 6.5 and 27.4 ± 0.4, respectively. Therefore, the distribution of BUP between the three compartments after 4 h is in the following order: tissue > maternal circuit > fetal circuit.

Other Factors Influencing Transplacental Transfer of BUP. There are several factors that can affect the concentration of “free” BUP and its transfer across the placenta, such as its binding to components of the perfusion medium, glassware, and type of tubing. To determine the effect of these factors, the tritiated opiate was recirculated in the model system in absence of a placenta. The decline in the concentration of [3H]BUP under these conditions was negligible, indicating that the opiate does not bind to the glass or tubing used.

Binding of [3H]BUP to dextran, a component of the perfusion medium (mol. wt. >40,000), was determined using gel filtration on desalting columns of Sephadex G-25. The amount of [3H]BUP appearing in the void volume of the column, i.e., bound to dextran, was 1.5 ± 0.1% of the opiate added to the medium. The remainder of the free drug was eluted at the total volume of the column. These data indicate that the decline in BUP concentration in the maternal circuit was due to that transferred to the fetal circuit or sequestered by the tissue only.

maternal artery and vein. These data suggest BUP but not AP has been retained by the perfused lobule/tissue.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Antipyrine (20 μg/ml)</th>
<th>Buprenorphine (10 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (min)</td>
<td>2.73 ± 0.48</td>
<td>5.83 ± 2.34</td>
</tr>
<tr>
<td>Fetal transfer rate (%)</td>
<td>41.9 ± 7.64</td>
<td>11.65 ± 2.50</td>
</tr>
<tr>
<td>Clearance (ml/min)</td>
<td>1.05 ± 0.12</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>Clearance index</td>
<td>0.277 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The level of BUP in the fetal circulation can have direct effects on the fetus, whereas that in the maternal may affect placental physiology, thereby indirectly affecting the fetus also. Information on the direct and indirect effects of BUP cannot be obtained from in vivo investigations due to ethical and safety considerations. However, the ex vivo technique/model system of dual perfusion of placental lobule has proven a valuable tool for obtaining such information for numerous drugs used for treating the pregnant patient.

Data presented here, using this technique, provide information on the effects of BUP on the placenta, the kinetics for its transplacental transfer, and its metabolism by the tissue. Values for the viability parameters of the tissue perfused with BUP were within the range of those for our control placentas (Table 1) and those reported by others (Schneider, 1995). Net fetal oxygen transfer was consistent with the diffusion of small molecules from the maternal to the fetal circulation. A slight increase in the release of the hormone was observed with the increase in BUP concentration but was pronounced and statistically significant (p < 0.01) at its concentration of 30 ng/ml or 60.0 nM (Table 1). This stimulation of hCG release is in agreement with that reported on the effect of other high-affinity opiate agonists and peptides on trophoblast tissue explant cultures (Cemerikic et al., 1991, 1993). Accordingly, the binding constant for BUP to placental κ-opiate receptors was determined and revealed high-affinity, with a \( K_d \) of 0.83 ± 0.23 nM. The wide range in κ-receptors densities, with a \( B_{max} \) of 79.0 ± 25.0 fmol/mg of protein, reflects its interplacental variability (Ahmed et al., 1986). Therefore, the observed stimulation of hCG by BUP provides evidence for the retention of a placental physiological function not affected by the opiate. The stimulation of hCG release may be of importance in view of preliminary reports on its role as an inhibitor of human immunodeficiency virus-1 infection in transgenic mice and placental explant cultures (Polliotti et al., 2000; Rao, 2000). Accordingly, it can be speculated that treatment of the pregnant opiate addict, who contracted the virus, with BUP may offer the advantage of decreasing/eliminating the viral load. Taken together, BUP at its concentrations tested may have no indirect effects on fetal growth and development.

The direct effects of BUP on the fetus depend on concentration of the opiate in its circulation. Data reported here indicate that less than 10% of the BUP dose was transferred to the fetal circuit and most of the remainder was retained by the tissue (Fig. 2B). The very low transplacental transfer of BUP is further illustrated by a fetal/maternal AUC ratio of 0.29 ± 0.07 at the end of the experiment compared with a ratio of 0.95 ± 0.06 for AP, which attained equilibrium within 2 h and was accompanied by its semiquantitative transfer to the fetal circuit (Fig. 2A). The sequestering of BUP by placental tissue after 4 h of perfusion was confirmed by its concentration ratios in tissue/maternal and tissue/fetal of 13.1 ± 6.5 and 27.6 ± 4.0, respectively. Taken together, it can be concluded that the initial transplacental transfer of BUP to the fetal circuit, although rapid, is minimal because most of the opiate is sequestered by the tissue. These findings are in agreement with the two-step process explaining transplacental transfer of highly lipophilic drugs in which the 1st step is uptake of the drug by the syncytiotrophoblast from the maternal circulation and the second is its transfer from the tissue to the fetal circulation (Sastry, 1999).

The elimination of BUP was investigated in experiments where the fetal circuit simulated “sink” conditions (open). The concentration-time curve for BUP in the maternal circuit was biphasic, exhibiting a distribution period during which the concentration of the opiate declined rapidly in the maternal circuit and was followed by an elimination phase during which a very shallow decline in the concentration of the opiate with a prolonged \( t_{1/2} \) of 5 to 6 h was observed. These data indicate a slow release of the drug from the tissue followed by a slow elimination. A similar profile for the concentration-time curve of BUP in the fetal circuit was also observed. Under the same experimental conditions, the terminal part of the elimination phase for AP in the maternal and fetal circuits showed a continuous decline in its concentrations with a \( t_{1/2} \) of 2 h.

Taken together, it can be concluded that the lipophilic property of BUP causes its distribution into the tissue and decreases its levels in the maternal and fetal circuits. These in vitro data are consistent with the pharmacokinetic profile of BUP after its sublingual and buccal administration, which indicated extended elimination half-lives and was attributed to the depot effect of tissues (Kuhlman et al., 1996).

The data presented here suggest that placental tissue retains BUP and raises a question on its subsequent fate. The period after transfusion of BUP and retention by the tissue in vitro can simulate that between two doses of the drug administered to a pregnant patient within a period of 48 h. Accordingly, BUP and AP were transfused for 2 h to “load” the tissue with the drugs followed by a period of another 2 h during which the tissue was perfused with medium only (washing period). The data obtained indicated that AP concentrations in the two circuits declined very rapidly and the drug was not detected in either circuit after 40 min (Fig. 4A). On the other hand, BUP, which attained a concentration of 67.02 ± 7.03 ng/g after 2 h of perfusion, was slowly released in both circuits during the washing period and was 40.0 ± 7.4 ng/g of tissue at the end of the experiment, i.e., 40% of the drug was released from the tissue within 2 h (Fig. 4B). Whether a similar release of BUP from placental tissue after its accumulation occurs in vivo is unclear but is likely to be true. Therefore, we may extrapolate our findings and suggest that the majority of BUP administered in vivo may be sequestered by the placenta then is slowly released in the maternal and fetal circulations during the following period of 2 days until the next dose of the drug is administered (three times per week regimen). If so, then a decrease in fetal exposure to the opiate and consequently the risk for, and or severity of, neonatal abstinence syndrome should also be observed. Indeed, data in four reports on 26 newborns to mothers maintained on BUP during pregnancy were examined and revealed that 15% required treatment, 19% did not require treatment, and 65% did not show any signs of neonatal withdrawal symptoms (Marquet et al., 1997; Fischer et al., 1998, 2000; Regini et al., 1998).

The biotransformation of BUP by human placental tissue and the distribution of norBUP between the tissue, maternal, and fetal circuits was determined. Because the opiate is transfused into the placental intervillous space, it is not accessible to the metabolic enzymes present in the maternal myometrium and endometrium and consequently the
amounts of metabolite formed should be less than that in vivo. Our data indicate that less than 5% BUP was metabolized to norBUP and was distributed between the maternal, fetal, and tissue compartments. On the other hand, in adult human liver, BUP is metabolized to norBUP by cytochrome P450 and CYP3A4 is responsible for 75% of this oxidative N-dealkylation. The remainder 25% are metabolized by yet to be identified enzyme(s) (Iribarne et al., 1997; Kobayashi et al., 1998). The enzyme(s) catalyzing the N-dealkylation of BUP to norBUP in term placenta is yet to be identified. To the best of our knowledge, CYP3A4 mRNA has been identified in the 1st and 3rd trimester placenta but the amount of its protein was extremely low (Hakkola et al., 1996,a,b). It is unclear whether our data on the low biotransformation potential of BUP by placental tissue is due to CYP3A4 and or the other yet to be identified enzyme reported in the liver. Therefore, it can be concluded that the metabolism of 5% or less of BUP during perfusion is a minimum and should be higher in vivo specially if the responsible enzyme(s) is induced due to the "chronic" administration of the opiate during pregnancy.

In summary, therapeutic concentrations of BUP in maternal serum appear to have no in vitro adverse effects on placental tissue viability and functional parameters, and consequently the opiate may have no indirect effects on the fetus. Placental tissue acts as a depot for BUP and renders its transplacental transfer to the fetal circuit very low. Therefore, the direct effects of BUP on the fetus will depend on its levels in its circulation and, in view of the limited reports available, appear to cause mild to nonexistent withdrawal symptoms in the newborns of mothers treated with the opiate.

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