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Transfer of L-α-Acetylmethadol (LAAM) and L-α-Acetyl-*N*-normethadol (norLAAM) by the Perfused Human Placental Lobule

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ABSTRACT

The agonists buprenorphine and L-α-acetylmethadol (LAAM) were introduced as alternatives to methadone for treatment of the adult opiate addict. The direct and indirect effects of these drugs on normal fetal growth and development are currently under investigation in our laboratory. The goal of this report is to provide part of the data necessary to assess the safety of LAAM in treatment of the pregnant opiate addict. To achieve this goal, the technique of dual perfusion of placental lobule was utilized to determine the kinetics for transplacental transfer of LAAM and its effects on the viability and functional parameters of the tissue. LAAM is rapidly metabolized to the pharmacologically active norLAAM that was also included in this investigation. The two opiates were transfused at their plasma levels in patients under treatment, a concentration of 35 ng/ml. The drugs exhibited similar pharmacokinetic profiles, characterized by an initial phase of distribution into placental tissue followed by their low transfer to the fetal circuit. During the 4-h experimental period, the transfused tissue retained significant amounts of LAAM and norLAAM, and neither drug was metabolized. LAAM did not affect placental tissue viability and functional parameters. However, norLAAM caused a significant decrease in the release of human chorionic gonadotropin. At this time, it is unclear whether a similar effect for norLAAM may occur in vivo and, if so, what the consequences would be on its role in implantation and normal fetal growth and development.

Methadone has been the only drug available for treatment of the adult opiate addict until buprenorphine (BUP) and L-α-acetylmethadol (LAAM) were introduced. Methadone has also been used in treatment of the pregnant opiate addict for several decades and became the standard of care for this patient population. Reports on clinical trials for treatment of the latter patient population with BUP showed that it was well tolerated by the woman, and neonatal abstinence syndrome was mild to nonexistent (Fischer et al., 2000). A recent report from our laboratory indicated that BUP, in the concentration range tested, did not have any effect on placental tissue viability and functional parameters, and its transfer to the fetal circulation was very low (Nanovskaya et al., 2002). On the other hand, LAAM has been used for treatment of the adult opiate addict since 1993. However, to the best of our knowledge, reports on its safety and/or use in treatment of the pregnant opiate addict have not been made. Results of controlled clinical trials indicated that LAAM is as effective as methadone in reducing illicit heroin use in the adult patient (Eissenberg et al., 1997; Oliveto et al., 1998). The effectiveness, efficacy, cost of use, safety, advantages, and drawbacks of LAAM in the treatment of the adult patient were the subject of many reports and review articles, including Prendergast et al. (1995) and Finn et al. (1997).

LAAM is a synthetic analog of methadone with long-acting μ-agonist properties. It has a slow rate of elimination, allowing its dosing three times per week and hence increasing patient compliance.

LAAM undergoes extensive and rapid first-pass metabolism by two demethylation reactions to L-α-acetyl-*N*-normethadol (norLAAM) and levo-α-acetyl-*N*,*N*-dinormethadol (dinorLAAM), leading many to consider the parent compound as a prodrug (Billings et al., 1973). LAAM and norLAAM are predominantly metabolized in human liver and intestine by microsomal cytochrome P450 3A4 (Moody et

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ABBREVIATIONS: BUP, buprenorphine; LAAM, levo-α-acetylmethadol; norLAAM, levo-α-acetyl-*N*-normethadol, dinorLAAM, levo-α-acetyl-*N*,*N*-dinormethadol; AP, antipyrine; TR_f, fetal transfer rate; CF_v, venous fetal concentration; CM_a, arterial maternal concentration; CL_m, maternal clearance; Q_f, fetal perfusion rate; CL_{index}, clearance index; AUC, area under the concentration-time curve; hCG, human chorionic gonadotropin; HPLC, high-performance liquid chromatography.

al., 1997; Neff and Moody, 2001; Oda and Kharasch, 2001a,b). In vivo and in vitro studies revealed that norLAAM possesses greater opiate activity and longer duration of action than LAAM, whereas dinorLAAM is less active than the parent compound (Nickander et al., 1974; Smits, 1974; Horng et al., 1976; Walczak et al., 1981; Vaupel and Jasinski, 1997). Plasma levels of norLAAM and dinorLAAM remain higher for longer periods of time than those for methadone, thus delaying the onset of withdrawal symptoms (Blaine et al., 1981).

A drug administered to a pregnant woman may cross the placenta to the fetal circulation and, depending on its concentration, could cause direct pharmacological or toxic effects to the fetus. Alternatively, the drug may affect placental functions, thus causing an indirect effect on the fetus. Therefore, to assess the potential of LAAM in treatment of the pregnant woman, it is crucial to determine its direct and indirect effects, which are impossible to obtain from in vivo investigations due to safety and ethical considerations. Also, there are no data from animal models on the effects of LAAM during pregnancy, and it should be noted that they could not be extrapolated to humans because of the fundamental differences in placental anatomy and functions.

The goal of this investigation is to determine the concentration of LAAM and norLAAM in the fetal circulation as well as their effects on the viability and functions of placental tissue. To achieve this goal, the technique of dual perfusion of term human placental lobule was utilized.

Materials and Methods

Dual Perfusion of Placental Lobule. Experimental details of the technique as used in our laboratory (Nanovskaya et al., 2002) were identical to those described by Miller et al. (1993). Briefly, each placenta was obtained from a full-term uncomplicated pregnancy and examined for tears. Vessels supplying a single peripheral cotyledon were cannulated with umbilical catheters, and the selected lobule was placed, maternal side up, in the perfusion chamber. Perfusion was initiated by inserting two catheters into the intervillous space on the maternal side. The perfusate was made of tissue culture medium M 199 (Sigma-Aldrich, St. Louis, MO) supplemented with Dextran 40 (7.5 g/l in the maternal and 30 g/l in the fetal perfusate), 1 g/l glucose, 25 IU/ml heparin, 40 mg/l gentamicin sulfate, and sulfamethoxazole and trimethoprim, 80 and 16 mg/l, respectively. The fetal perfusate was equilibrated with 95% N_2, 5% CO_2 and the maternal with a mixture of 55% O_2, 5% CO_2, and the balance nitrogen. A solution of 7.5% (w/v) sodium bicarbonate was used to adjust the pH to 7.4. The temperature of the perfusates and the chamber was maintained at 37°C. The fetal and maternal flow rates were 2.8 to 3.2 and 10 to 12 ml/min, respectively.

Experimental Protocol. Each placenta was perfused for an initial period of 2 h in the absence of the opiate (control period) to evaluate the physical integrity of the tissue. The experiment was terminated if one or more of the following was observed: the pressure in fetal artery exceeded 50 mm Hg, a loss of >2 ml/h, or a difference between the fetal vein and artery <60 mm Hg (inadequate perfusion overlap between the two circuits). During the initial control period (2 h), 250-μl samples were taken from the maternal reservoir every 30 min to determine the viability and functional parameters and to establish its baseline levels for each placenta/experiment. Subsequently, the experimental period was initiated by replacing the perfusion medium in both the maternal (250 ml) and the fetal (150 ml) circuits with fresh medium and 8.75 μg of either LAAM or norLAAM added to the maternal reservoir, thus achieving a final concentration of 35 ng/ml. Antipyrine (AP; 20 μg/ml) was used as a marker for the transfer of an inert compound across the placental tissue and as a reference to account for interplacental variations. The radioactive isotopes [^{3}H]LAAM, 38.71 Ci/mmol, and [^{3}H]norLAAM, 6.68 Ci/mmol, were prepared by the Research Triangle Institute and provided to us as a gift from the National Institute on Drug Abuse. [^{14}C]AP, 9.3 mCi/mmol, was purchased from Sigma-Aldrich. Each radioactive isotope was added to the maternal reservoir (1.5 μCi) to increase the detection limits of the compounds and allow the simultaneous determination of the opiate and AP by scintillation counting. Samples were collected at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, and 240 min from both the maternal and fetal circuits. The concentration of LAAM, norLAAM, and AP was determined in 0.5-ml aliquots of the perfusates, using a liquid scintillation analyzer (PerkinElmer Life Sciences, Boston, MA). The results obtained represented the transfer of free drugs because no protein (e.g., albumin) was added to the perfusates.

Transplacental Transfer and Distribution of LAAM and norLAAM. The amount of LAAM and norLAAM added to the maternal reservoir results in a final concentration of 35 ng/ml. This concentration of the two opiates was chosen because it is similar to that reported for their plasma levels in patients receiving a dose of 20 to 40 mg of LAAM (Walsh et al., 1998). In all experiments reported here, this concentration of LAAM and norLAAM was used irrespective of the type of perfusion system utilized. The latter was dictated by the kinetics parameter to be determined.

Open-Open System (Both the Maternal and Fetal Circuits Are Not Recirculated). This type was utilized to determine the transfer rates of LAAM and norLAAM under steady state conditions and is considered most suitable for comparing the pharmacokinetic parameters of different compounds. The transfer parameters were calculated according to the following equations (Bourget et al., 1995): fetal transfer rate (TR_f) = [CF_v/CM_a] × 100%, where CF_v and CM_a are venous fetal and arterial maternal concentrations of the opiates; maternal clearance (CL_m) = (CF_v/CM_a) × Q_f (ml/min), where Q_f is the fetal perfusion rate; clearance index (CL_{index}) = TR_f opiate/TR_f AP.

Closed-Open System (The Maternal Circuit Only Is Recirculated). This type was utilized to determine the elimination half-life ($t_{1/2}$) and the elimination rate constant. The concentration-time curve (AUC) for the maternal circuit was analyzed by nonlinear regression with noncompartmental method (PCNONLIN software; Statistical Consultant Inc., Lexington, KY).

Closed-Closed System (The Maternal and Fetal Circuits Are Recirculated). This type is considered the closest simulation of in vivo conditions and was utilized to determine the distribution of the opiates between the tissue, maternal, and fetal circuits. At the end of each experiment, the perfused region of the tissue (white in color) was dissected, weighed, and homogenized in saline. An aliquot of the homogenate (1 ml) was incubated in the dark with 1 ml of 1 M NaOH for 12 h at 60°C. Scintillation fluid (8 ml) was then added and the radioactivity determined. At the end of each experiment, 0.5-ml aliquots from the maternal and fetal reservoirs were obtained, and the radioactivity of the remaining opiate was determined by liquid scintillation spectrometry.

Concentration of the Free Drugs. The binding of [^{3}H]LAAM and [^{3}H]norLAAM to dextran was determined by gel filtration on desalting columns of Sephadex G-25. The data obtained indicated that the amounts of [^{3}H]LAAM and [^{3}H]norLAAM bound to dextran were negligible (less than 0.5%).

The Effects of LAAM and norLAAM on Tissue Viability and Functional Parameters. Samples of 250 μl were collected from the maternal reservoir every 30 min and centrifuged at 1000*g* for 10 min at 4°C; the supernatant was stored at −70°C until the concentrations of glucose, lactate, and human chorionic gonadotropin (hCG) were determined as described earlier (Nanovskaya et al., 2002). The values obtained from these assays were used to assess placental tissue viability and function. During the experiment, samples were also collected every 30 min from both circuits and analyzed immediately

for their content of pH, pO_2, and pCO_2 using a blood gas analyzer (model 1620; Instrumentation Laboratory, Milan, Italy). The values recorded during the experimental period were compared with those of baseline levels established during the control period as well as for the same time points (total of 6 h) in control placentas perfused under identical conditions but without the addition of any drugs. The viability and normal physiological functions of the perfused lobule were validated by its consumption of glucose and oxygen, lactate production, and release of the hormone hCG. Our laboratory has established a range of values for the above-mentioned parameters in control placentas that are in agreement with those of other laboratories; namely, glucose consumption and lactate release were between 0.18 to 0.68 and 0.20 to 0.77 μmol/min · g, respectively. The values for oxygen consumption depended on the thickness of the tissue and lobule size (Schneider, 1995).

Metabolism of LAAM and norLAAM. Tritiated LAAM and norLAAM are (−)-[1,2-^{3}H]α-acetylmethadol and acetyl-*N*-normethadol. The enzyme-catalyzed demethylation of LAAM to norLAAM or the latter to dinorLAAM yields products that retain their tritiated atoms. Extraction of LAAM and norLAAM from placental tissue, the maternal and fetal perfusates, was carried out as described by Moody et al. (1997). Briefly, the two opiates were extracted with *n*-butyl chloride, and the organic layer was evaporated by a steam of nitrogen at 40°C. The dried residue was dissolved in 0.5 ml of the mobile phase utilized in high-performance liquid chromatography (HPLC). The mobile phase was methanol/water (80:20, v/v) containing 3.5 g/l $(NH_4)_2CO_3$ and 0.1% triethylamine. The stationary phase was a C_{18} column, and the opiates were eluted at a flow rate of 1 ml/min and monitored at a wavelength of 224 nm. The HPLC system used was a Varian Star system (Varian Inc., Palo Alto, CA) consisting of a Varian 9021 solvent module, an autosampler (model 9095), and a UV-visible detector connected in series with a Schoeffer Instruments model 970 fluorescence detector (McPherson Inc., Chelmsford, MA) attached to a Hewlett-Packard model 3395 integrator. Fractions of 1 ml were collected from the system and analyzed for their radioactivity using a liquid scintillation spectrometer. Retention times for LAAM and norLAAM standards were determined under identical conditions. The specific activity of LAAM and norLAAM under the experimental conditions used were 620 and 390 dpm/ng, respectively, which allowed high detection limits of any metabolite formed during the 4-h experimental period.

Statistical Analysis. All values reported are expressed as mean ± S.D. Statistical significance of the differences observed between LAAM and norLAAM groups and between the control and experimental periods was 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.

Results

Transplacental Transfer of LAAM and norLAAM. The technique of dual perfusion of term human placental lobule was used in three of its types to determine the kinetics for transplacental transfer of the opiates and their distribution into the fetoplacental unit, as well as their effects on the viability and functional parameters of the tissue. The value reported for each viability or functional parameter is the mean for those obtained from several experiments carried out on the corresponding number of placentas and is indicated in the figure legends.

Open-Open System. This type of dual perfusion was utilized to determine the transfer rates of LAAM and norLAAM under steady-state conditions. Antipyrine was cotransfused with each of the two opiates to normalize the transfer rates and account for interplacental variations. The pharmacokinetic profile for AP in this type of perfusion system was described in detail in a previous report from our laboratory (Nanovskaya et al., 2002).

In each experiment, either LAAM or norLAAM was added to the maternal reservoir to achieve a final concentration of 35 ng/ml. Under the steady-state conditions of an open-open system, the concentrations of LAAM and norLAAM in the maternal artery were 34.5 ± 1.43 and 35.9 ± 2.15 ng/ml, respectively. It is apparent from Fig. 1, A and B, that LAAM and norLAAM were transferred from the maternal artery across the placental tissue to the fetal vein within the initial 10 min of their transfusion. The lag time for LAAM was 7.1 ± 1.7 and for norLAAM 6.8 ± 1.4 min. During the following 50 min, the concentrations of LAAM and norLAAM in the fetal circuit exhibited a steady increase, reached a maximum after 90 min for the former and 60 min for the latter, and remained constant until the end of the experimental period of 2 h. The fetal transfer rates for the two opiates during the latter period of time were noticeably different, 17.4 ± 6% for LAAM and 26.9 ± 7% for norLAAM. Upon normalization of these values with the transfer rate of AP in each placenta, the difference between the resulting clearance indexes of the two opiates reached statistical significance ($p < 0.05$; Table 1), indicating that the amount of norLAAM transferred to the fetal circuit is higher than that for LAAM.

Closed-Open System. Transfusion of LAAM and norLAAM in this type of system creates sink conditions in the fetal circuit. These conditions resemble in vivo elimination of the drugs assuming that they are metabolized and or excreted by the fetoplacental unit. AP was cotransfused with each opiate as a reference compound.

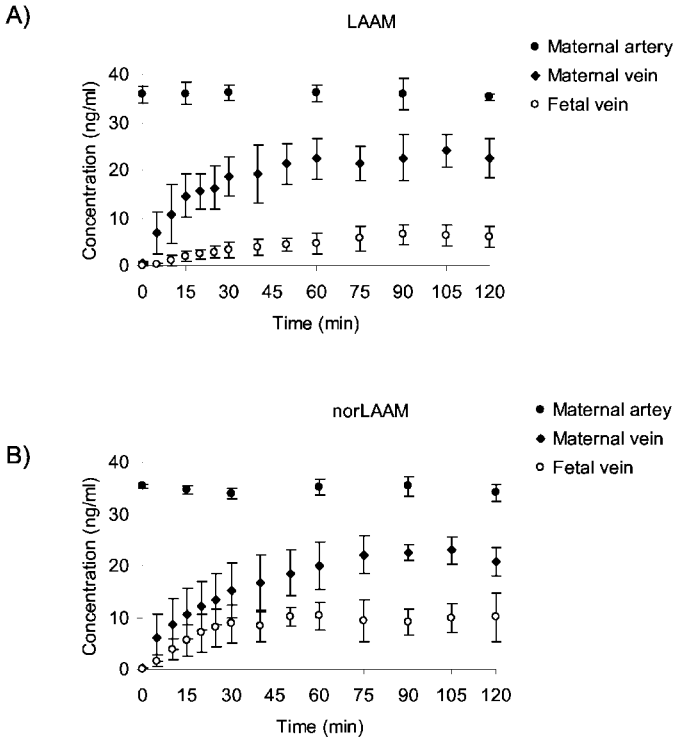

Fig. 1. Concentrations of LAAM (A) and norLAAM (B) in the maternal and fetal circuits of a placental lobule perfused under steady-state conditions (open-open) for 2 h. LAAM and norLAAM (n = 4 placentas for each) crossed the placenta rapidly, achieving fetal transfer rates of 17.4 ± 6 and 26.9 ± 7% after 90 min and 60 min, respectively.

TABLE 1
The pharmacokinetic parameters for LAAM and norLAAM in an open-open system
Values are the mean for four placentas ± S.D.

Parameters	LAAM	norLAAM
Lag time (min)	7.15 ± 1.75	6.81 ± 1.46
Fetal transfer rate (%)	17.4 ± 5.80	26.9 ± 6.77
Clearance (ml/min)	0.531 ± 0.13	0.821 ± 0.18
Clearance index	0.492 ± 0.11	0.799 ± 0.07*

* $p < 0.05$.

During the initial 60 min following the transfusion of either opiate, a steep decline in its concentration in the maternal artery was observed and was more rapid than that for AP. During the second hour of perfusion, the decline in the concentration of AP continued at approximately the same rate, whereas that for the opiates became much slower, reaching a plateau during the last 2 h of the experiment. It is interesting to note that the continuous decline in the concentration of AP in the fetal vein is in agreement with that expected for sink conditions. However, the slow decline in the concentration of the two opiates in both the fetal vein and maternal artery can be explained by their release from the placental tissue. It is apparent that the decline in the concentration of LAAM and norLAAM in the maternal circuit exhibited a biphasic pattern, rapid during the initial 60 min (distribution phase), then very shallow thereafter (elimination phase, Fig. 2, B and C). Our calculations revealed a mean half-life ($t_{1/2}$) of 458 ± 93.8 min and an elimination rate constant (k_{el}) of 1.5 ± 0.4 h^{-1} for LAAM. For norLAAM, the $t_{1/2}$ was 357 ± 52.0 min and the k_{el} was 2.0 ± 0.3 h^{-1}. In contrast, the $t_{1/2}$ for AP was 154 ± 29.9 min and the k_{el} was 4.6 ± 1.0 h^{-1}. These data suggest that placental tissue act as a depot for the opiate during its initial phase of distribution, i.e., shortly after administration; then, the drug is slowly released into the circulation.

Closed-Closed System. Researchers in the field agree that using the technique of dual perfusion of placental lobule with recirculation of both perfusates is the closest available simulation of in vivo conditions. Other advantages for the closed-closed system include determining the kinetics for distribution of the drug between the tissue and the two circuits as well as accumulation of the metabolites formed during the experimental period. In each experiment, either LAAM or norLAAM was added to the maternal reservoir, to achieve a final concentration of 35 ng/ml.

It is apparent from Fig. 3, A and B, that the decline in the concentration of LAAM and norLAAM in the maternal artery is biphasic, rapid in the initial 30 min and slower during the following second phase until equilibrium is reached. It should be noted that the time needed to attain equilibrium was <60 min for norLAAM and 90 min for LAAM, and the difference is likely due to their lipophilic properties.

It was also apparent from our data that there was a rapid drop in the initial amounts of LAAM and norLAAM in the

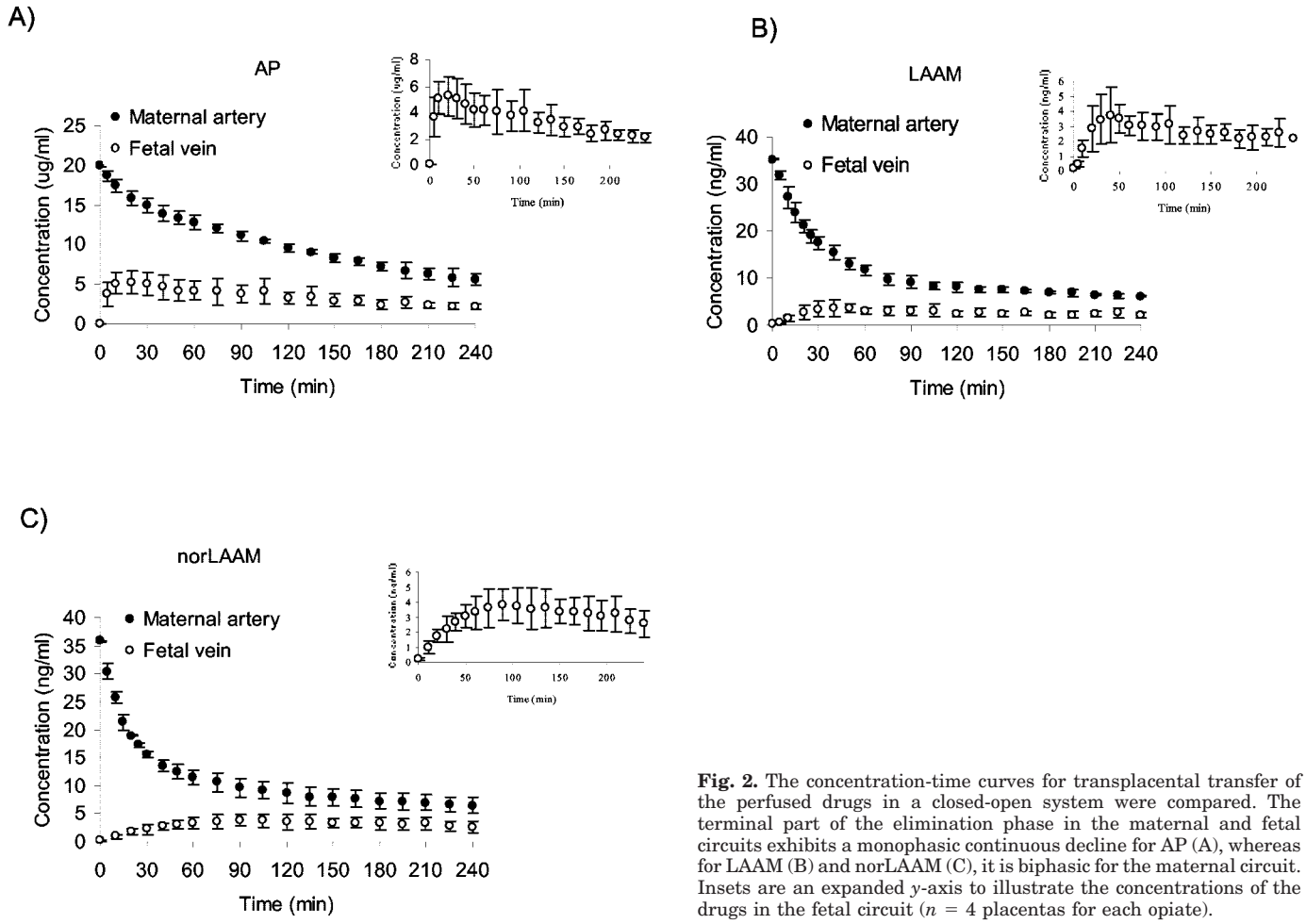

Fig. 2. The concentration-time curves for transplacental transfer of the perfused drugs in a closed-open system were compared. The terminal part of the elimination phase in the maternal and fetal circuits exhibits a monophasic continuous decline for AP (A), whereas for LAAM (B) and norLAAM (C), it is biphasic for the maternal circuit. Insets are an expanded y-axis to illustrate the concentrations of the drugs in the fetal circuit (n = 4 placentas for each opiate).

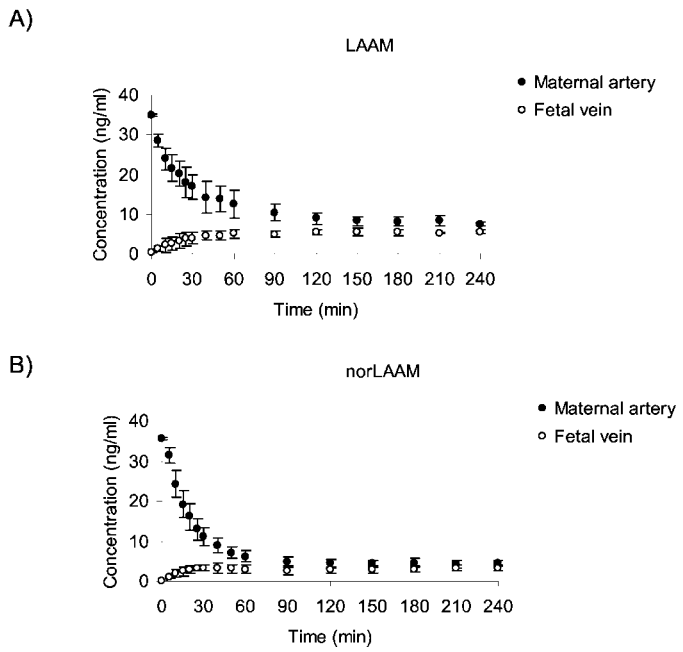

Fig. 3. Transfer of LAAM (A) and norLAAM (B) across human placenta in a closed-closed system during 4 h of perfusion. A rapid decline (50%) in the concentration of the opiates in the initial 30 min of the transfusion was not accompanied by their appearance in the fetal circuit, suggesting their retention by placental tissue (n = 6 placentas for LAAM and 5 for norLAAM).

maternal artery. The drop was very noticeable and corresponded to 51.5 ± 12.1% of the initial concentration for LAAM and 65.1 ± 4.6% for norLAAM. It is also very clear that the amounts of opiates disappearing from the maternal circuit were not observed in the fetal circuit, i.e., no quantitative transplacental transfer. A similar observation was made earlier in our laboratory for BUP and was then compared with the decline in the concentration of AP from the maternal circuit that was simultaneously accompanied by its quantitative appearance in the fetal circuit (Nanovskaya et al., 2002). For LAAM, the amount transferred to the fetal circuit represented 8.9 ± 2.4%, whereas that retained by the placental tissue corresponded to the remaining 42.6 ± 9.6%. Similarly, the distribution of norLAAM was 54.9 ± 4.7% in the tissue and the remainder 10.1 ± 2.0% in the fetal vein.

These data indicate that the administration of either opiate is followed by a period of rapid uptake and accumulation by the placenta. It appears that the placenta retains most (80%) of the opiate lost from the maternal circuit, and the remainder (20%) is transferred to the fetal circuit.

The Effects of LAAM and norLAAM. The viability and functional parameters of a dually perfused placental lobule were determined in control experiments. These biochemical parameters were determined during a 6-h perfusion period in a placenta every 3 weeks, and the range for the data obtained represent the "control" values for our laboratory (see *Materials and Methods*). In addition, the initial 2-h period of each experiment was a control for a specific placenta, and the values obtained for the viability and functional parameters represent the baseline for the perfused lobule; i.e., each placenta acts as its own control. Therefore, any changes in the baseline values of the viability and functional parameters during transfusion of the opiates would represent their effects. The viability parameters are oxygen delivery, transfer, and consumption; glucose utilization; and lactate production. The parameter for placental function is hCG release.

Transfusion of LAAM did not affect oxygen delivery, transfer, and consumption during the experimental period. This finding indicates that each placenta had adjusted to the ex vivo conditions during the control period, had adequate oxygen supply during the control and experimental periods, and was not affected by the transfused opiates (Table 2).

The effect of LAAM on glucose utilization, lactate production, and hCG release indicated a downward trend with time and was consistent with that for control placentas (Table 3). These data suggest that placental tissue exposed to LAAM for 4 h was not adversely affected.

The effects of norLAAM on the viability and functional parameters of the tissue indicated that it did not affect oxygen delivery, transfer, and consumption despite the observed decrease in the values of the latter. However, its effects on glucose utilization and lactate production during the third and fourth hours of perfusion were pronounced, and the difference in their values from control was statistically significant. These data indicate that the opiate has affected the tissue's metabolic activity. In addition, the statistically significant decrease in hCG release during the transfusion of norLAAM suggests that at least one of the tissue's physiological functions may have been adversely affected (Fig. 4). Taken together, these data indicate that norLAAM had ad-

TABLE 2

Effects of LAAM and norLAAM on oxygen delivery, transfer, and consumption during the 4-h experimental period relative to the initial 2-h control period (no opiates)

Each value represents the mean of at least six placentas ± S.D. The values for the initial 2-h control period were set as 100%.

	O_2 Delivery		O_2 Transfer		O_2 Consumption	
	Experimental Period	Percentage of Control Period	Experimental Period	Percentage of Control Period	Experimental Period	Percentage of Control Period
	ml/min · kg		*ml/min · kg*		*ml/min · kg*	
LAAM						
60 min	12.51 ± 4.56	96	0.405 ± 0.11	107	4.74 ± 2.67	88
120 min	13.17 ± 2.49	101	0.389 ± 0.16	103	4.96 ± 0.68	91
180 min	12.55 ± 3.21	96	0.354 ± 0.02	94	5.27 ± 1.45	97
240 min	13.78 ± 3.39	106	0.413 ± 0.01	109	5.48 ± 2.57	101
norLAAM						
60 min	12.41 ± 5.25	97	0.450 ± 0.21	88	3.44 ± 1.13	75
120 min	13.16 ± 5051	103	0.364 ± 0.22	71	3.88 ± 1.75	84
180 min	12.70 ± 4.16	99	0.482 ± 0.21	94	3.47 ± 1.25	75
240 min	12.17 ± 4.01	95	0.468 ± 0.10	91	3.15 ± 1.69	68

TABLE 3
Effect of LAAM and norLAAM on the viability parameters of the tissue during the 4-h experimental period relative to the initial 2-h control period
Each value represents the mean of at least six placentas ± S.D. The values for the initial 2-h control period were set as 100%.

	Glucose Consumption		Lactate Production	
	Experimental Period	Percentage of Control Period	Experimental Period	Percentage of Control Period
	$\mu mol/min \cdot g$		$\mu mol/min \cdot g$	
LAAM				
60 min	0.278 ± 0.16	95	0.364 ± 0.16	97
120 min	0.231 ± 0.09	78	0.321 ± 0.12	86
180 min	0.229 ± 0.14	77	0.338 ± 0.14	83
240 min	0.210 ± 0.13	70	0.298 ± 0.10	80
norLAAM				
60 min	0.391 ± 0.11	103	0.365 ± 0.12	91
120 min	0.341 ± 0.13	90	0.339 ± 0.07	84
180 min	0.228 ± 0.09*	60	0.261 ± 0.11*	65
240 min	0.205 ± 0.06*	54	0.230 ± 0.09*	57

* $p < 0.05$.

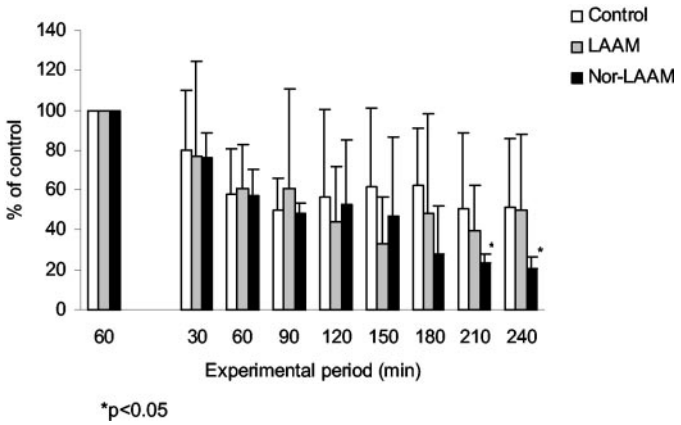

Fig. 4. The effects of LAAM and norLAAM on the rate of hCG release from trophoblast tissue (n = 6 placentas for each). The rate of hCG release during the experimental period was expressed as a percentage of that during the control period, which was set at 100%. A statically significant decrease in the release of hCG was observed in the placentas perfused with norLAAM.

verse effects on the tissue's viability and functional parameters.

Metabolism of LAAM and norLAAM. Each opiate at its final concentration of 35 ng/ml in the maternal reservoir was cotransfused with its tritiated isotope (1.5 μCi) in a closed-closed system to allow accumulation of the formed metabolite and maximize its detection limit. Identification of the metabolites formed was achieved by their retention times on an HPLC C-18 column. The retention times for standards of LAAM, norLAAM, and dinorLAAM under the experimental conditions described under *Materials and Methods* were 19, 10, and 7 min, respectively.

In experiments/placentas in which LAAM was transfused for 4 h, norLAAM and dinorLAAM were not detected in the tissue, or maternal or fetal circuits. Also, when norLAAM was transfused, dinorLAAM was not detected. Since the tritium atom of the transfused isotope is retained in the formed metabolite, and because of the lack of its detection by scintillation spectrometry in the HPLC fractions corresponding to the retention times of their standards, it can be concluded that the tissue does not metabolize the opiates during 4 h of perfusion.

Discussion

Currently, the drugs available for treatment of the adult opiate addict are methadone, BUP, and LAAM. However, pregnancy can occur while a woman is abusing opiates or under treatment for addiction. Methadone has been the only drug available for treatment of the pregnant opiate addict during the last five decades. Data on its successes and limitations were the subject of numerous reports and review articles. More recently, reports on the use of BUP in clinical trials for treatment of the pregnant opiate addict were made. Comparisons between maternal and neonatal outcome of women treated with BUP and methadone indicated that the former might have advantages over the latter.

LAAM is the other alternative to methadone that has been used for treatment of the adult opiate addict since 1993 (Jones et al., 1998; Johnson et al., 2000). However, to the best of our knowledge, reports on the use of LAAM in treatment of the pregnant woman are scarce to nonexistent. In addition, data on its direct and indirect effects on normal fetal growth and development are lacking. The goal of this investigation was to provide part of the information needed to assess the safety of LAAM in treating the pregnant opiate addict. To achieve this goal, the concentrations of LAAM and norLAAM in the fetoplacental unit and their effects on placental tissue were determined by utilizing the technique of dual perfusion of term human placental lobule.

The long-acting properties of LAAM are based on the pharmacology of its two metabolites (norLAAM and dinorLAAM). norLAAM is 15 to 200 times more potent than LAAM in binding assays (Nickander et al., 1974) and 6 to 12 times more potent in vivo (Vaupel and Jasinski, 1997). In contrast, in vivo investigations of dinorLAAM suggested that it is less potent than LAAM (Smits, 1974; Walczak et al., 1981). However, dinorLAAM was reported as being 1.5 to 3 times more potent than LAAM in binding assays (Nickander et al., 1974). The rapid demethylation of LAAM during its first-pass metabolism led several investigators to consider it as a prodrug. Therefore, it was equally important to determine the concentration of norLAAM in the fetal circuit and its effects on the tissue.

LAAM and norLAAM were added to the maternal reservoir to achieve a final concentration of (35 ng/ml), i.e., comparable to their concentration in the adult patients after administra-

tion of a therapeutic dose (20–40 mg) of LAAM (Walsh et al., 1998).

Transplacental transfer of a compound can be due to its intrinsic properties, e.g., partition coefficient in oil, or extrinsic properties, such as placental age or type of transport across a biological membrane, i.e., by passive, active, or facilitated diffusion. Passive diffusion depends on the flow rate of the solute on both sides of the membrane (flow-limited), while either active or facilitated diffusion depends on the presence of a selective transporter protein in the biological membrane (membrane-limited). In the experiments cited in this report, the ratio of maternal to fetal flow rate is 4.4 as compared to the in vivo ratio of 5.7.

Other factors influencing drug transfer in the ex vivo system used are placement of the catheters into the intervillous space to simulate the maternal circuit as well as its degree of overlap with the fetal circuit. To normalize the data obtained from each experiment and account for interplacental variations, the inert marker compound antipyrine was cotransfused with each opiate in every experiment.

Placental transfer of compounds from maternal to fetal circulation is a two-stage process: 1) uptake by the trophoblast tissue and 2) transfer from the tissue to the fetal circulation (Sastry, 1999). It is interesting to note that transfer of the less lipophilic compound norLAAM (log P, 3.90) to fetal circulation in our experiments was higher than that for LAAM (log P, 4.27). This finding is in agreement with our previous observation that the fetal transfer rate of the most lipophilic compound, BUP (log P, 5.10), was only 11% (Nanovskaya et al., 2002).

An examination of the pharmacokinetic profiles for LAAM and norLAAM indicated similarities in their rates of distribution and elimination. During the initial phase of distribution, there was a rapid decline in the concentration of each opiate in the maternal circuit. In the following elimination phase, the decline in their concentration was very shallow (Fig. 2, B and C). It should be noted that the initial sharp decline in concentration of the opiates was not accompanied by their appearance in the fetal circuit, in contradistinction to AP, which was detected in the latter immediately (Fig. 2A). In addition, the elimination constants for LAAM (k_{el} = 1.5 h^{-1}) and norLAAM (k_{el} = 2.0 h^{-1}) were significantly ($p < 0.01$) lower than that for AP (k_{el} = 4.6 h^{-1}). Taken together, these data indicate that the opiates were rapidly distributed into, and sequestered by, the placental tissue and, subsequently, slowly released in the maternal and fetal circuits.

The role of the placenta as a depot for LAAM and norLAAM is illustrated by the ratio of the drug sequestered by the tissue to that in the maternal and fetal circuits. These ratios were: for LAAM, tissue/maternal, 15.8 ± 1.7, and tissue/fetal, 20.2 ± 4.6; and for norLAAM, tissue/maternal, 18.2 ± 7.7, and tissue/fetal, 24.6 ± 9.04. It can be speculated, on basis of these data, that upon administration of LAAM to a pregnant addict, hepatic enzymes will biotransform the drug rapidly to norLAAM (first-pass metabolism), and subsequently, the placental tissue would retain and accumulate both opiates. It should be mentioned here that human placenta undergoes maturation and structural changes during gestation, and data obtained from a model system utilizing the tissue at term may not be extrapolated to earlier periods.

In general, drugs administered during pregnancy could have an adverse effect on placental viability and/or physiological functions, and, consequently, an indirect effect on fetal development. In the ex vivo model system utilized, the consumption of oxygen and glucose, as well as the production of lactate, serves as an indicator for the effects of LAAM and norLAAM on the normal metabolic activity of the tissue. Conversely, the release of hCG during the experimental period, as compared with the control, is used as an indicator of placental function. These viability and functional parameters were not affected by the transfusion of 35 ng/ml LAAM for a period of 4 h, indicating that LAAM does not have any detectable adverse effects on placental tissue.

In contrast, norLAAM caused a decrease in the rates of glucose utilization and hCG release during the third and fourth hours of its transfusion. The effect of norLAAM on hCG release was more pronounced (20% of the control). Earlier reports have established that there is a very wide range for the amounts of hCG released by individual placentas both in vivo (Alfthan and Stenman, 1990) and in vitro (Cemerikic et al., 1991); i.e., the amount of the hormone released is an inherent property of the placenta. Accordingly, the amounts of hCG released during transfusion of the opiates were expressed as a percentage of that during the last hour of the control period. At this time, it is difficult to speculate on whether the observed effects of norLAAM in the ex vivo model system could occur in vivo and, if so, whether their effects will be more or less pronounced.

The metabolism of LAAM and norLAAM by a placental lobule during their perfusion for 4 h was investigated. In experiments in which either LAAM or norLAAM was transfused for 4 h, their respective metabolites, norLAAM and dinorLAAM, were not detected at the nanogram level. However, the lack of detectable metabolic activity for the tissue under our experimental conditions cannot be extrapolated to the in vivo conditions, since both opiates would have greater access to the placental metabolic enzymes in the latter (Wiegand et al., 1984).

In summary, the transfusion of LAAM at a concentration comparable to its peak plasma levels, following administration of a therapeutic dose, does not seem to adversely affect placental tissue viability and functional parameters. Placental tissue retains most (80%) of the administered LAAM, and only a fraction (20%) is transferred to the fetal circuit. Conversely, LAAM is biotransformed in vivo to norLAAM by first-pass metabolism, leading to higher serum levels of the latter than the parent compound. Therefore, it is likely that placental tissue would be exposed to increasing concentrations of norLAAM following the administration of a therapeutic dose of LAAM. Data obtained in this investigation indicate that the fetal transfer rate of norLAAM is almost twice that of LAAM, and the former can have adverse effects on the placental viability and functional parameters as determined in the ex vivo system used. Taken together, the results indicate that it is likely that administration of LAAM to a pregnant woman would result in higher concentrations of norLAAM than LAAM in placental tissue and in the fetal circulation. It is unclear at this time whether the observed adverse effects for norLAAM on placental tissue would also occur in vivo.

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